Role of peptide modifications in differentially modulating multiple components of a rhythmic neuromuscular system in the American lobster, *Homarus americanus*

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Central pattern generators (CPGs) are neural networks that control rhythmic behaviors, such as breathing, walking, and chewing. In order to produce a wide variety of behaviors in response to different environmental stimuli, CPGs must have flexibility in their electrical output. This flexibility can be achieved through peptidergic modulation. Peptides are short strings of amino acids that act as signaling molecules in the nervous system, allowing for cellular communication. Encoded in longer sequences, peptides are enzymatically cleaved within the neurosecretory pathway and may undergo further post-translational modification (PTMs) before becoming fully bioactive. These modifications regulate how peptides interact with the cellular environment, ensuring stability and proper bioactivity. When bound to their receptor, peptides can alter neuronal function and elicit unique behaviors.

The goal of this project was to further elucidate the molecular underpinnings of peptidergic modulation through physiological experiments combined with transcriptomics and molecular biology. The CPG that drives the rhythmic contractions of the lobster heart, the cardiac ganglion (CG), serves as an excellent model for studying neuromodulation. The CG is a relatively simple network composed of only nine neurons that produces quantifiable output that responds to peptide modulators. One such modulator is myosuppressin (pQDLDHVFLRFamide). Previous research in the Dickinson lab (Stevens, et al., 2009) has shown that, when applied to whole hearts, myosuppressin causes an immediate decrease in contraction frequency and contraction amplitude, followed by a large increase in contraction amplitude. By applying myosuppressin to isolated CGs and externally stimulated muscle cells, it was found that myosuppressin acts both centrally, on the neurons themselves, and peripherally, at the neuromuscular junction and/or muscles. It was found that myosuppressin alters a number of the burst characteristics of the CG, causing the decrease in contraction frequency and amplitude and contributing to the delayed increase in amplitude. However, changes at the periphery of the system also independently contributed to the increase in amplitude. One explanation for these observed effects is that there are multiple myosuppressin receptors that are differentially distributed within the CG and muscle. To address this possibility, this summer I mined previously generated tissue-specific transcriptomes to predict the presence of myosuppressin receptors in the CG and muscle. Five myosuppressin receptors were predicted, three of which exist in the CG.

In addition, it has been shown that myosuppressin exists endogenously in the lobster with two PTMs: a cyclization of the (N)-terminal glutamine and amidation at the (C)-terminus. It may also exist in forms that lack certain modifications, which may be differentially bioactive. My previous research tested three isoforms of myosuppressin on whole hearts of lobsters; one isoform with both modifications, one isoform lacking the cyclization, and one isoform lacking amidation. I found that both the fully modified isoform, and the non-cyclized isoform were able to elicit a decrease in frequency and a decrease in amplitude followed by a large increase. In contrast, the non-amidated isoform was able to elicit a smaller decrease in frequency and amplitude, but was not able to elicit an increase in amplitude. This summer I investigated whether the differentially modified isoforms of myosuppressin were able to elicit a response in the periphery of the system, in the absence of neuronal input. To do this, I dissected out the cells of the CG and provided manual stimulation at a nerve ending to evoke contractions of the heart. In this experiment, the fully modified form of myosuppressin and the non-cyclized form caused an increase in the amplitude of nerve-evoked contractions, whereas the non-amidated form caused no change in the amplitude of contractions, suggesting that the non-amidated isoform is unable to bind either at the neuromuscular junction or muscle.

To further investigate the binding capabilities of myosuppressin, I began to characterize the expression of the predicted receptor sequences in the neural tissues of the lobster. Using real time PCR, four predicted myosuppressin sequences were identified in the CG, two of which were also identified in the muscle. One receptor was identified in the muscle that was not identified in any CG samples. Future research will confirm the sequences of the predicted receptors and express the sequences in an insect cell line to test their ability to bind, and perhaps selectively bind, to differentially modified isoforms of myosuppressin.

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References

Stevens, J. S., Cashman, C. R., Smith, C. M., Beale, K. M., Towle, D. W., Christie, A. E., & Dickinson, P. S. (2009). The peptide hormone pQDLDHVFLRFamide (crustacean myosuppressin) modulates the *Homarus americanus* cardiac neuromuscular system at multiple sites. *Journal of Experimental Biology, 212*(24), 3961-3976. doi:10.1242/jeb.035741.