Examining the Distribution of the C-type Allatostatins (C-AST) and C-AST-like peptides in the lobster *Homarus americanus*
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The allatostatins are pleiotropic arthropod neuropeptides that are known to inhibit juvenile hormones in insects. Currently, there are three known families of allatostatins: A-type, B-type and C-type allatostatins (ASTs). C-AST, which is characterized by the non-amidated C-terminal motif –PISCF, a pyroglutamine blocked N-terminus and a disulfide bridge between the Cys residues at position 7 and 14, had been found and predicted only in holometabolous insects (Stay and Tobe, 2007). A recent study using transcriptomics and mass spectrometry, however, has identified C-AST in decapod crustaceans (Stemmler et al. 2010). In addition, another study using similar methods has identified an AST-C-like peptide (AST-C-LP) that also has a disulfide bridge between the two Cys residues in decapod crustaceans (Dickinson et al. 2009). Despite their identification, little is known about the cellular distribution or the physiological effects of the two peptides in these species.

The Dickinson lab has previously examined the effects of C-AST and AST-C-like peptides on the heart of the American lobster, *Homarus americanus*. Although the two peptides are commonly hypothesized to have similar bioactivities in a given species by acting upon the same set of receptors in cells (Veenstra 2009), the Dickinson lab observed that this was not always the case. In all of their lobsters, the perfusion of both C-AST and AST-C-like peptides consistently led to a decrease in the heartbeat frequency. In a subgroup of their samples, however, C-AST decreased the amplitude of the heartbeat while AST-C-LP increased it in the same animal. Given this disparity, the researchers have raised the question about the extent to which these two peptides are regulated together or independently.

To address this question, my project focused on identifying the distribution of the two peptides in parts of the stomatogastric nervous system (STNS) of the lobster, which is a commonly studied nervous system to understand the neuromodulatory control of a small neural circuit in the crustacean species. If my results showed that C-AST and AST-C-like peptides resided in different neurons within the STNS, they would provide support for the hypothesis that the two peptides can be released separately. To examine the distribution, I performed immunohistochemistry using primary antibodies raised against C-AST-LP. My results revealed that AST-C-LP was extensively distributed throughout the stomatogastric nervous system, including ~15 somata and a neuropil in the commissural ganglia and 2 somata and a neuropil in the stomatogastric ganglion. Since I was examining two structurally similar peptides, it was essential to ensure that the antibodies recognize only their own antigen, i.e., AST-C-LP. Incubation of the antibodies with an excess of AST-C-LP before the incubation of tissues completely eliminated staining, while incubation of the antibodies with an excess of C-AST had no effect on the staining. Therefore, I could be confident that the staining I observed was due to the presence of AST-C-LP, and not to cross-binding with C-AST. My future work will focus on determining the specificity of antibodies raised against C-AST and performing double-labeling experiments to compare and contrast the distributions of C-AST and AST-C-LP in the same animal.
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References:

