Mechanisms for producing different chewing patterns in the crab: SIFamide as a potential neurotransmitter of the modulatory commissural neuron 5 (MCN5)

Linna Gao, 2013

Crabs are important marine organisms that inhabit the rocky and sandy coasts of Maine in abundant numbers. Amongst the common crabs in Maine are two species of Cancer crabs, Cancer irroratus and Cancer borealis. Like most decapods crustaceans, crabs are highly opportunistic feeders and depend on their ability to eat and digest a wide range of food in order to survive. Due to the wide variety of foods that crustaceans eat—ranging from decaying (therefore soft) fish to lobster (with the shell still intact), there is a substantial need for the ability to produce a wide array of patterns so that the animal can effectively chew its food. The stomatogastric nervous system (STNS) is the part of the crustacean nervous system that controls the foregut, in which chewing and the initial processing of food takes place. Most of the neurons in the stomatogastric ganglion are attributed to the pyloric filter, which divides food particles by size, and the gastric mill, which controls the grinding of the three teeth in the crab stomach. Both of these rhythmic networks are considered to be Central Pattern Generators (CPGs), which are fixed networks of nerves that generate the neuronal outputs that drive rhythmic movements. These networks are vitally versatile and able to create a wide variety of outputs that allow animals to react to their intrinsic and extrinsic environments. Modulation of these networks, mostly by peptides and amines, allows for flexibility in the patterns that these networks generate (Dickinson, 2008). Many studies have ascribed this flexibility to the actions of locally-released and circulating peptides on the STNS neural circuits. Subsequently, the physiological identification and characterization of the projection neurons that provide the local modulatory inputs to the stomatogastric ganglion (STG) of crab Cancer borealis has been the focus of a large amount of research.

Numerous peptides have been identified in crustaceans, including the SIFamides, a family of arthropod brain and gut peptides. In the crabs Cancer borealis and Cancer irroratus, GYRKPPFNGSIFamide (G-SIFamide) has been confirmed to be the native isoform of the peptide (Stemmler et al., 2007). Although a significant amount of research has focused on determining their structural conservation in decapods, there is not much known about the neuronal distributions of the SIFamides or about the functional roles they serve. SIFamides have only been physiologically examined in one species, the American lobster Homarus americanus (Christie et al., 2006; Dickinson et al., 2008; Rhem et al., 2008). In the lobster, the peptide was shown to have an excitatory effect on the pyloric motor pattern. Recently, research has indicated that GYRKPPFNGSIFamide is present in the stomatogastric nervous system of Cancer crabs. Specifically, immunohistochemical evidence has also shown that one cell that stains for SIFamide has the same morphology as the modulatory commissural neuron 5 (MCN5) of the crab (unpublished, Andrew Christie, MDIBL). This suggests that GYRKPPFNGSIFamide may be a co-transmitter in the MCN5 neuron. The potential identification of SIFamide as a co-transmitter in MCN5 suggests a role for the peptide in modulation of the pyloric neural network, as MCN5 itself in the STNS works in conjunction with the pyloric pacemaker ensemble to excite an enhanced pyloric motor pattern rhythm (Norris et al., 1996). SIFamide peptide has already been shown to have an excitatory effect on the crab heart pattern, and preliminary data from the Dickinson lab shows that it has an activating effect on the pyloric pattern in the STNS. The goal of the
research I want to pursue this summer is to further examine the physiological actions of SIFamide on the crab stomatogastric ganglion. In addition to determining how it modulates this system, my data will help to test the hypothesis that SIFamide is the major modulatory neurotransmitter of the modulatory neuron MCN5.

This research was conducted using male and female crabs *Cancer borealis*, which were thoroughly anesthetized before dissection. The stomach was then be dissected, cut open ventrally and the stomatogastric nervous system was removed from the stomach by fine dissection under the microscope. The STNS was then superfused with physiological saline to keep it alive. The electrical outputs of nerves on the STNS was measured with electrodes that were placed inside vaseline wells surrounding a part of the nerves being recorded. The neuropeptide SIFamide was then be applied to the stomatogastric ganglion. This allowed me to compare the effects of SIFamide on the crab ganglion with the effects of stimulating the MCN5 neuron. Physiological data have shown that G-SIFamide does indeed evoke effects comparable to MCN5 activation, which enhances both the pyloric pattern and gastric pattern in the STNS. This suggests that G-SIFamide is a major co-neurotransmitter of MCN5, and that many of the effects of MCN5 may be mediated by G-SIFamide, which is presently the only identified transmitter in the neuron.

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