MODULATION OF THE CALLINECTES SAPIDUS CARDIAC SYSTEM BY SEROTONIN

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Introduction

In this study, we used immunohistochemistry to determine the distribution of the biogenic amine Serotonin (5-Hydroxytryptamine [5-HT]), in the central nervous system (CNS) of the blue crab, *Callinectes sapidus,* and examined the modulatory effects of 5-HT on the physiology of the crab heart. The CNS of crustaceans include structures like the brain, thoracic ganglion, pericardial organs and the cardiac ganglion (See Fig. 1). The cardiac ganglion within the heart of the blue crab acts as a simple central pattern generator (CPG) -effector system. The 9 neuron CPG sets the basic rhythm for the heart, which can then be modulated both neuronally (via 3 cardioregulatory fibers) and hormonally (via the pericardial organs). GABA, an inhibitory neurotransmitter, and dopamine, an excitatory neurotransmitter, are thought to be the neurotransmitters of two of the cardioregulatory fibers, and are both present in the pericardial organs. The neurotransmitter of the third fiber is unknown. Here we examined the neuroanatomical distribution of 5-HT-like immunoreactivity in the CNS of the blue crab in an effort to determine the neurotransmitter of the third cardioreglatory fiber. Likewise, we performed perfused working heart trials to examine the effect of 5-HT on heart function to determine what possible role this neurotransmitter plays within the physiology of the blue crab.

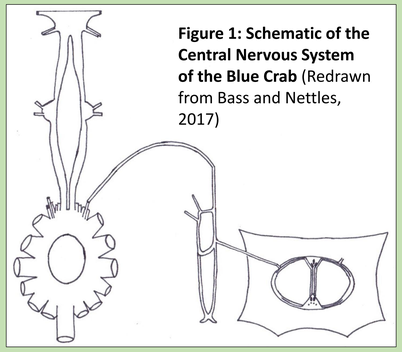


Figure 1: Schematic of the Central Nervous System of the Blue Crab (Redrawn from Bass and Nettles, 2017)

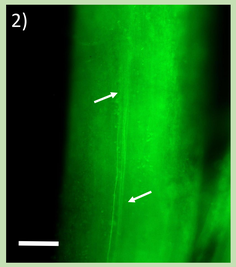


Figure 2: Brain-Thoracic Connective. Multiple fine caliber 5-HT-li reactive fibers in the core of the connectives (arrows). Bar = 200 µm.

Methods

Live blue crabs were purchased locally and maintained at Valdosta State University until use in aquaria. Crabs were placed under ice (a natural anesthetic) for 20 minutes.

Immunohistochemistry: The dorsal carapace was removed and the thoracic ganglia, brain, pericardial organs and cardiac ganglia were dissected and secured to Sylgard-lined petri dishes with minuten pins. Standard immunohistochemical protocols (Fort *et al*, 2004) were followed. Briefly, preparations were fixed for 4 hours in 4% paraformaldehyde. Preparations were washed (5X, 20 minutes / wash at room temperature with agitation) with phosphate-triton azide (PTA). After a 12 hour preincubation period with normal goat serum buffer (NGS) (10:800 NGS: PTA), samples were immersed for 48 hours in a primary antibody (Rabbit Anti- 5-HT (1:100 – 1:200 dilution) or Mouse Anti- 5-HT (1:50 dilution)). PTA washes followed (5X, 20 min., room temperature with agitation). Preparations were then incubated in secondary antibody (1:2000 – 1:5000) conjugated to a fluorescent marker (Alexa 488 Goat Anti-Rabbit or Goat Anti-Mouse IgG) for 24 hours. PTA washes were then repeated (5X, 20 min., room temperature with agitation). Preparations were then examined under a Nikon 600 Eclipse microscope and photographed.

Perfused Working Heart: The dorsal carapace was removed and hearts were dissected. Functioning hearts mounted in a Sylgard-line Petri dish and cannulated with a modified syringe needle mounted within the dish. The heart was attached to the force plates of a Grass FT03 isometric force transducer and placed under a resting load. The heart was perfused with crab saline at a constant rate (2ml/min) and pressure. Perfusion rate and pressure were maintained when 5-HT trials were performed. Varying concentrations of 5-HT (10^{-10} M to 10^{-3} M) were tested with washes of crab saline in between each drug concentration.

Abstract

The heart of the blue crab, Callinectes sapidus, is a simple central pattern generator-effector system. In this study, we used immunohistochemistry to determine the distribution of the biogenic amine, Serotonin (5-Hydroxytryptamine) in the central nervous system of the blue crab. Serotonin-like immunoreactivity (5-HT-li) was observed in cells in the brain and thoracic ganglion and in fibers within the brain-thoracic connectives. 5-HT-li fibers projected via segmental nerve 1 towards the PO. Multiple branching fine caliber fibers and varicosities expressed 5-HT-li within the PO. 5-HT-li was not observed in the dorsal nerve or CG. Serotonin induced both positive chronotropic and inotropic effects in isolated heart preparations at concentrations consistent with hormonal signaling. The 5-HT-li distribution and cardiac responsiveness to serotonin both suggest a possible cardiomodulatory role for serotonin in the heart of the blue crab.

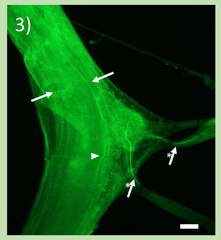
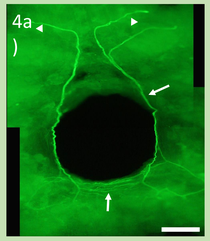


Figure 3: Commissural Ganglion. Medium caliber 5-HT-li reactive fiber projecting from the brain to the CoG (arrow). Medium caliber fiber projecting from the thoracic ganglion to the CoG (arrow head). 5-HT-li reactive fibers projecting from the CoG to the STG (arrow *). Bar = 200 µm.



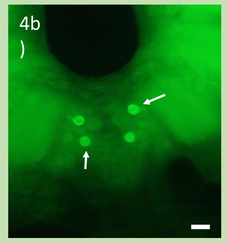


Figure 4: Thoracic Ganglion. (A) Large caliber 5-HT-li reactive fibers (rabbit polyclonal antibody) projecting from the posterior thoracic neuromere (arrows). Fibers branch and project towards the segmental nerves (arrow heads). (B) 4 large 5-HT-li reactive cells (mouse monoclonal antibody) in the posterior thoracic neuromere (arrows). Bars = 200 µm.

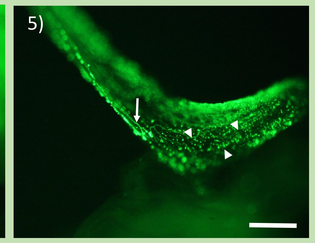


Figure 5: Pericardial Organ. Multiple fine caliber branching 5-HT-li reactive fibers (arrows) in the core of the PO with varicosities at the surface (arrow heads). Bar = 200 µm.

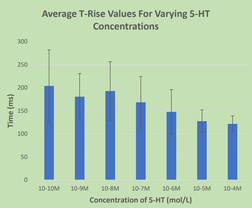


Fig. 6. Average T-Rise values (time from initiation of contraction to peak amplitude) for varying 5-HT concentrations. Time to peak decreased with increasing 5-HT concentrations indicating a faster rate of contraction.

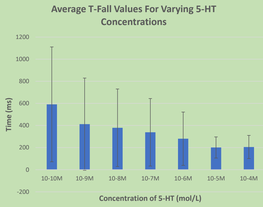


Fig. 7. Average T-Fall values (time from contraction to complete relaxation) for varying 5-HT concentrations. Time to complete relaxation decreased with increasing 5-HT concentrations indicating a faster rate of relaxation.

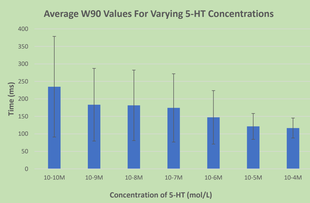


Fig. 8. Average W90 values (time for 90% of the contraction/relaxation cycle to occur) for varying concentrations of 5-HT. W90 time decreased with increasing 5-HT concentration.

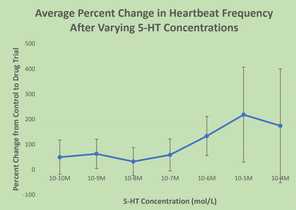


Fig. 9. Effect of 5-HT on heartbeat frequency. As 5-HT concentration increased, frequency increased. Effects of 5-HT on frequency were variable at low concentrations, that could be due to experimental error. Threshold for frequency effects were between 10^{-8}M and 10^{-7}M 5-HT

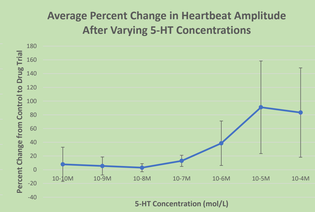


Fig. 5. Effect of 5-HT on heartbeat amplitude. As 5-HT concentration increased, amplitude increased. Effects of 5-HT on amplitude were less variable at low concentrations, that observed with frequency. Threshold for amplitude effects were between 10^{-8}M and 10^{-7}M 5-HT

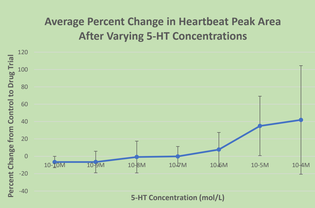


Fig. 6. Effect of 5-HT on heartbeat peak area (area under the curve). Average peak area increased with increasing 5-HT concentration, suggesting increased functional activity of each heartbeat.

Results

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5-HT-li was not observed in the brain, however there were multiple fine caliber fibers located in the brain-thoracic connectives. 2 medium caliber fibers in the connectives terminated with fine branches and varicosities around the cells of the commissural ganglia (CoG) (Fig. 7). 5-HT-li projections were observed arising from the CoG projecting to the stomatogastric ganglion (STG)(Fig. 7b). 4 large cells were seen in the posterior of the thoracic ganglia (Fig. 8b) with large caliber anterior projecting fibers that branched towards the segmental nerves (Fig 8a) In the POs, multiple fine caliber fibers branched throughout, with multiple varicosities observed at the surface (Fig. 9)

The analysis of the working heart preparations included data for frequency of heart beat, amplitude of contraction, peak area, time of rise (T-rise), time of decay (T-fall), and 90% duration of contraction cycle (W90). Average percent change from control values to each concentration of 5-HT was calculated for each of the variables. An increase in frequency (Fig.) and amplitude (Fig.) corresponded with increasing 5-HT concentrations, with a response threshold value occurring between 10^{-8} M and 10^{-7} M 5-HT. Increasing 5-HT concentration also led to an increase in peak area (Fig.). Average for T-rise, T-fall, and W90 were calculated. Increasing 5-HT concentration led to a general decrease in T-rise and T-fall was seen, indicating a faster rate of contraction and relaxation of the heart, respectively. (Figs. and). W90, time for 90% of the contraction/relaxation cycle to occur, also decreased with increasing drug concentration (Fig.).

Discussion

5-HT-li was observed widely throughout the CNS. The termination of 5-HT-li fibers in the CoG suggest Serotonin may modulate the cells of the CoG.

5-HT-li reactive cells in the posterior of the thoracic ganglia that project fibers towards the segmental nerves are the likely source of 5-HT-li in the POs. Further 5-HT-li immunohistochemistry of the CNS is required to resolve this.

The distribution of 5-HT-li (fibers and varicosities) in the pericardial organs suggest that the biogenic amine could be released as a neurohormone.

No 5-HT-li fibers were observed in the dorsal nerve (DN) or cardiac ganglion, which suggests that Serotonin is not the neurotransmitter of the third cardioregulatory fiber

5-HT induced increases in frequency, amplitude and peak contraction area at a threshold concentration consistent with the action of a neurohormone.

The decrease in T-rise, T-fall and W90 times all indicate a faster completion of each contraction/relaxation cycle, indicating quicker individual heartbeats.

The increased frequency, force and peak contraction area suggest an increased functional activity of the heart. Overall**,** this suggests that the amine could have an excitatory cardiomodulatory effect on the heart.

The data analysis of heart preparations exhibited high values for standard deviation. This was likely due to having a relatively small pool of data. Additional trials were prevented due to unforeseen circumstances. Further experimentation is required to acquire more consistent results and thus minimize the error.

References

Fort TJ, Brezina V, Miller MW. 2004. Modulation of an Integrated Central Pattern Generator-Effector System: Dopaminergic Regulation of Cardiac Activity in the Blue Crab *Callinectes sapidus*. Journal of Neurophysiology. 92(6):3455–3470.

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Acknowledgements

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