The Current Hunt for Nitric Oxide's Effects on the Lobster Cardiac Ganglion

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Abstract

A robust and rhythmic heartbeat is critical for the survival of animals. The heartbeat of the American lobster (*Homarus americanus*) is generated by the cardiac ganglion (CG), a central pattern generator consisting of small premotor and large motor neurons. Endogenous neuromodulators, such as nitric oxide, induce bursting flexibility in the CG to maintain homeostasis in the presence of environmental stressors. To investigate nitric oxide's inhibitory effects on the CG, I am using extracellular and two-electrode voltage clamp techniques to determine which cell populations and currents are altered to produce a resilient, rhythmic heartbeat. Preliminary results suggest NO decreases bursting frequency in the large cells to inhibit the CG. I expect future experiments will develop a broader understanding of flexibility in patterned movements.

Project Objectives

Central pattern generators are neural networks that drive and maintain rhythmic motor patterns independently of sensory input (Dickinson, 2006). In *Homarus americanus*, the CG consists of 4 small premotor cells (SC) and 5 large motor cells (LC). The electrical signaling from these coupled neurons ultimately generate a rhythmic heart contraction that is critical for sustaining physiological functions (Cooke, 2002).

Nitric oxide (NO), a gaseous signaling molecule, has an inhibitory role within the lobster CG (Mahadevan, 2004). A robust heartbeat is dependent on the negative feedback from NO on the CG (Mahadevan, 2004). The contraction of the heart leads to the release of NO, which decreases the bursting frequency of the CG. Consequently, the amplitude of heart muscle contractions decreases. Further investigating this neuromodulator can yield new insights into the mechanisms by which NO regulates the CG.

This summer project seeks to uncover which neurophysiological currents are responsible for the inhibitory effects of NO by recording from the isolated CG. Previous research has suggested NO activates a signaling cascade that involves cGMP (Scholz et al, 2002), but the specific currents that mediate the inhibition of bursting is still unknown. Learning about these undetermined currents will support the lab's broader objective of understanding the mechanisms that provide flexibility within patterned movements in the nervous system.

Methodology

Animals

American lobsters (*Homarus americanus*) were purchased from seafood stores in Brunswick, Maine, USA. Lobsters were kept in recirculating natural seawater aquaria (10-12°C) and fed shrimp or squid weekly. Before isolation of the heart, lobsters were anesthetized in ice for 30 min. The heart was dissected from the cephalothoracic carapace in chilled (8-10°C) physiological saline. The CG was isolated by ventrally cutting the heart and dissecting the main ganglion trunk from the surrounding muscle.

Extracellular Recordings

The CG was pinned to a Sylgard lined petri dish, and Vaseline wells were made around the anterolateral nerve (ALN) at LC 1 and the trunk at SC 6 (Fig. 1). One electrode was placed inside the well and the other outside in the bath for extracellular recordings. Saline was pumped through a cooling wand and

into the dish at 2.5 mL/min via a Rabbit peristaltic pump (Rainin). Two metal tubes suctioned the bath solution out to perfuse saline across the preparation. A liquid cooling system (Koolance) and temperature controller (Warner Instruments) maintained a constant temperature of 10-12˚C.

A suture silk fiber (0.1 mm) was tied around the CG trunk anterior to LC 4 to separate the small and large cells (Fig 1). Tightening this ligature caused only small cell spike recordings from the trunk and only large cell spikes on the ALN.

Nitric Oxide and SNAP

The CG was exposed to NO by perfusing SNAP (10^{-5} M), a NO donor, across the preparation for 10-15 minutes, followed by a 30-45 minute saline wash. The wash ensured that the preparation remained sensitive to NO modulation. A preparation continued with the extracellular or intracellular protocol if a preliminary ~20% decrease in burst frequency was induced by nitric oxide (Fig. 2).

Intracellular Recordings

After dissecting the whole heart from the lobster, the CG was isolated and pinned to a Sylgard lined petri dish. Two glass microelectrodes with squid intracellular fill (8-13 MΩ) were then inserted into a single LC for the two-electrode voltage clamp technique, which records the ionic currents flowing across the cell membrane. The CG was perfused with saline, nitric oxide, and a cocktail of channel blockers to isolate different ionic currents. The signals were then amplified and analyzed.

Results

Preliminary extracellular recordings showed SNAP had a significant effect on the LCs but no effect on SCs. The burst frequency of ligatured LCs significantly decreased after SNAP application (paired t-test, $p<0.05$, n=6, Fig. 3a). The burst duration and duty cycle of the LCs, as well as the measured bursting parameters of the SCs, were not significantly affected by SNAP application (Fig. 3b,c).

Intracellular recordings confirmed the effects of SNAP in one of the large cells, where a qualitative decrease in burst frequency was identified (Fig. 4). SNAP application did not cause a clear change in burst duration or duty cycle, further suggesting that NO does not affect the burst duration of LCs.

Discussion

Nitric oxide induces negative feedback within the CG to provide a robust heartbeat. Mahadevan (2004) found that heart muscle contraction amplitude was decreased by NO. My results showed a decrease in the bursting frequency of LCs after SNAP application, elucidating how muscle contraction amplitude may be modulated. By inhibiting the LCs, the downstream musculature output also becomes inhibited. In addition to the unaffected LC bursting duration and duty cycle, the SC bursting parameters were not significantly modulated by NO. The burst frequency, burst duration, and duty cycle of the SCs were not significantly affected by NO, but the variability between preparations may be concealing NO's effects. An increased sample size may uncover NO effects and highlight the role of SCs in the negative feedback of nitric oxide.

Intracellular recordings of one of the large cells also showed a decreased burst frequency and constant burst duration. These results suggest an increased hyperpolarizing current or decreased depolarizing current that increases the inter-burst interval. The mechanisms by which nitric oxide inhibits bursting has not yet been uncovered. Through future experiments with two-electrode voltage clamp techniques, I hope to isolate the specific cellular properties that are modulated by NO.

Figures

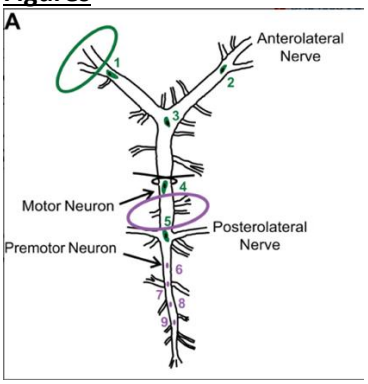

Figure 1. The cardiac ganglion of the *Homarus americanus* contains 4 small cells and 5 large cells. The small cells (purple) are premotor neurons that drive bursting patterns of large cells. The large cells (green) are motor neurons that drive heart muscle activity. A ligature placed anterior to large cell 4 decouples small and large cells for individual bursting recordings. The circled purple and green ovals indicate recording sites for, respectively, small cells in the trunk and the large cells in the anterolateral nerve.

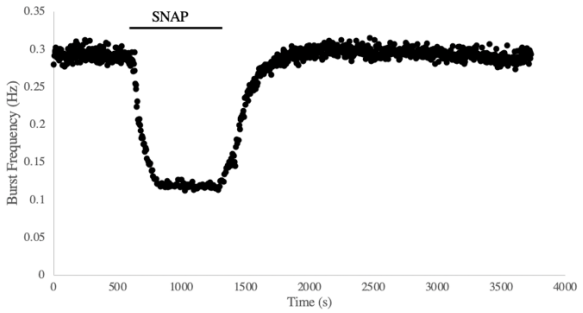

Figure 2. Time course of SNAP application in a control large cell illustrates the effect of SNAP on burst frequency. Perfusion of SNAP over the CG for 10 minutes (indicated by line) led to decrease in burst frequency. The acute exposure to SNAP also allowed for the effects to be washed out by saline (30-45 minutes).

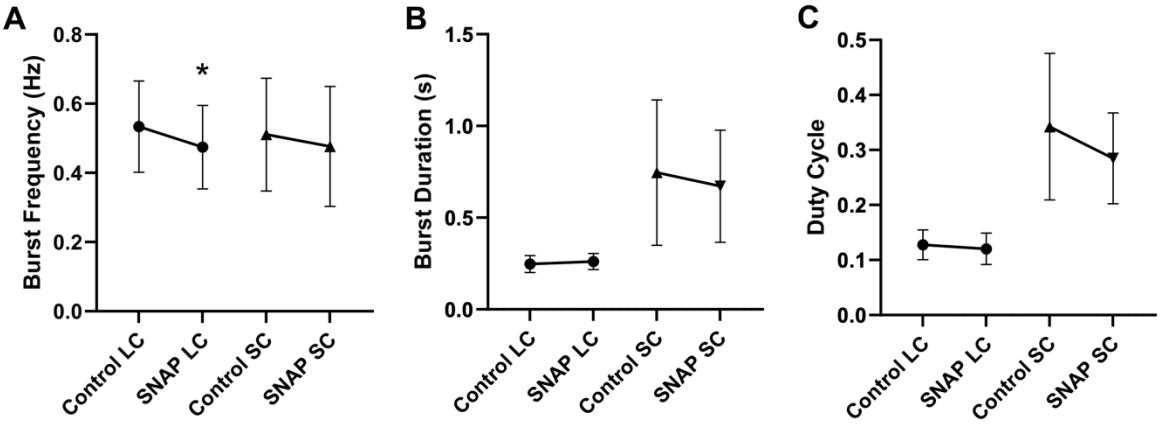

Figure 3. SNAP application significantly decreases burst frequency of the ligatured LC. The LC burst frequency decreased by 0.059 Hz ($p=0.0046$, two-tailed paired t-test, $n=6$). However, the burst frequency of the SC, in addition to the burst duration and duty cycle of both cell populations, did not significantly change (95% confidence interval).

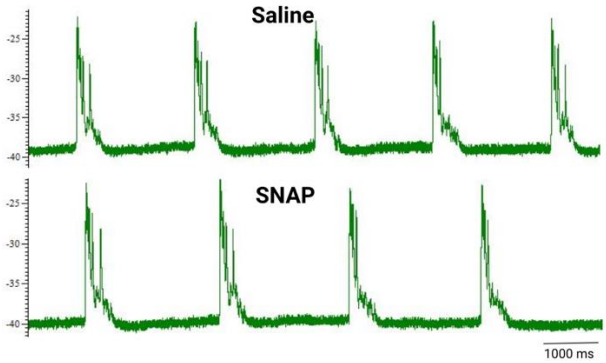

Figure 4. Model of two-electrode voltage clamp recording of the effects of SNAP on one of the large cells. Intracellular recordings indicate SNAP application decreases burst frequency but has no clear effect on burst duration. The interburst interval may be increased due to an increased hyperpolarizing current or decreased depolarizing current.

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