The Modulation of Calcium-activated Potassium Channels for Stabilizing Mammalian Spinal Locomotor Activity

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I investigated the pharmacological effects of blocking and enhancing calcium-activated potassium channels and the greater response that this modulation has on the overall motor activity produced by the mouse spinal cord. The mouse model closely resembles that of humans in terms of locomotion processes, which is the rhythmic pattern of motor activity relating to motor and sensory processes. What drives the ability to perform locomotion is a biological neural system known as the central pattern generator (CPG), which is a neural circuit that produces rhythmic outputs in the absence of sensory or other brain-related input. CPGs generate repetitive, cyclic activity that underlies the motor operations of locomotion, and so I looked at how modulating calcium-activated potassium channels regulates the bursting motor activity of the CPG and the excitability of the neural network.

Neural CPG networks rely on iconic currents to mediate their activity, and the ionic current that I focused on was the calcium-activated potassium channel. There are two major types of calcium-activated potassium channels, BK (large-conductance) channels and SK (small-conductance) channels. Both BK and SK channels play a role in the regulation of neuronal excitability and neuronal firing potentials by affecting the afterhyperpolarization (AHP) phase of the action potential. As a continuation of the project I started last summer, we looked to block and enhance BK and SK channels, both in isolation and in combination with one another. Apamin was used as the SK channel blocker and SKA-31 as the SK channel activator. Iberiotoxin was used to selectively block BK channels and NS1619 was used as the BK channel activator.

The modulation of SK and BK channels have been explored on a neuronal level in previous literature, but the effects on the CPG network level were absent, which is what my research aimed to fulfill. Last summer, applying SK and BK channel blockers was found to result in a reduced AHP phase, meaning that there was a greater frequency of action potential firing and an overall increase in neural network excitability. This summer I categorized the action potentials of the control data into three speeds – fast, normal, slow. The hypothesis was that when channel blockers were applied for slow rhythms, the bursting activity will stabilize and the three motor bursting parameters will differ: higher burst amplitude (the height of the burst), shorter burst duration (the onset to offset of a neuronal burst), and shorter cycle period (the time between the onset of a burst to the beginning of a new burst).

Methods: We worked with a neonatal (1-5 day old) rodent model *in vitro* lumbar spinal cord preparation. The spinal cord was dissected via a ventral laminectomy and placed in a recording chamber where it was perfused with a mouse normal ringer solution. We activated the lumbar CPG neuronal network with a combination of NMDA and 5-HT to record the motor neuron axonal firing of the rhythmic bursting. Once rhythmic bursting was achieved (termed "fictive locomotion") by either two contralateral (opposite side) or ipsilateral (same side) ventral roots that cause alternating rhythm, we then perfused SK and BK channel blockers to test their effects. We had six experimental conditions: apamin (100nM), iberiotoxin (100nM), and apamin+iberiotoxin (100nM of each), CyPPA (5uM), NS1619 (5uM), and CyPPA+NS1619 (5uM of each). We conducted a 20-minute control treatment, followed by a 45-minute drug treatment, and finished with a 60-minute wash treatment. We then analyzed the data by rectifying and smoothing with a custom script to measure the amplitude, burst duration, and cycle period of motor neuron bursting.

Results: As both channel blockers apamin and iberiotoxin decreases the refractory period duration, this induces increased excitation of the spinal locomotor rhythm. From the flexor L2 ventral root, apamin significantly increased the peak amplitude as compared to the control and had no significant difference for the burst duration or cycle period. Iberiotoxin decreased the peak amplitude, burst duration, and cycle period. The apamin+iberiotoxin combined trial increased the peak amplitude and had no significant difference for the burst duration or cycle period. The results were largely similar for the extensor L5 root. When the enhancers were applied to a fast rhythm to modulate the over-excited neural network to stabilize, we saw indications of decreased network excitability. When CyPPA was applied to the extensor

L5 root, the peak amplitude decreased, the burst duration and cycle period increased. The NS1619 had no significant difference in the peak amplitude but saw an increase in burst duration and cycle period.

Future Experiments: We will work towards a dose-response curve in the future to measure what concentrations of the activators/blockers are most effective in modulating a disrupted rhythm. We also will work towards increasing the n of the different drug treatments to boserve a more representative analysis. Thirdly, we want to measure our data with circular statistics for a better representation of the variability in rhythms, moving away from fast, normal, slow to how disruptive or stable the rhythm is in the control data set.

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