Dopaminergic Modulation of HCN1 Channels as a Potential Mechanism Underlying Hyperarousal in RLS

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Restless Leg Syndrome (RLS) is a neurological sensorimotor disorder characterized by a self-reported urge to move and periodic leg movements during sleep (PLMS).[1, 2] This hyperactivity has been linked to a decreased availability of adenosine A_1 receptors (A1Rs).[3] In a healthy individual, these receptors work to inhibit excitatory neurotransmission by forming functional heteromers or pairs with dopamine D_1 receptors (D1R) in the lumbar region of the spinal cord; thus, decreased A1R availability and the resulting lack of A1/D1 heteromers results in a disinhibited or hyperdopaminergic transmission pathway within the central nervous system, creating the restlessness exhibited by RLS patients.[4] D1R activation has been shown to modulate HCN1 channels by increasing the intracellular concentration of the second messenger cAMP.[5] HCN1 channels carry the hyperpolarization-activated current (I_h), an inward cation current that primary serves to return a neuron to its resting potential and often is involved in burst initiation. My summer research project aimed to establish the importance of I_h in mammalian locomotion under hyperdopaminergic conditions mimicking those seen in RLS patients. I hypothesized that blocking the HCN1 channels would result in an increase in cycle period and lead to a less stable rhythm.

To test this hypothesis, I isolated the spinal cords of six neonatal Swiss Webster mice one to five days after birth using a ventral laminectomy procedure. The neonatal mouse is a common animal model for locomotion research as the neural circuit that controls walking behaviors, called a central pattern generator, is like that of humans and can be neurochemically activated.[6] I then used glass micro suction electrodes to record the extracellular activity of ventral roots L2 and L5, which indicate extensor and flexor muscle contractions respectively, to assess burst amplitude, burst duration, and cycle period. Fictive locomotion, defined as alternating activity between opposite L2/L5 nerves (interlimb alternation) and between same-side L2 and L5 nerves (intralimb alternation), was induced using serotonin (5HT; 9-15μM) and N-methyl-D-aspartate (NMDA; 6μM) before adding dopamine (DA; 50μM) to determine its effects. I then used the HCN1 blocker ZD7288 (1μM) to investigate the role of I_h in mouse locomotor-like activity.

I found that blocking HCN1 channels caused a significant increase in all three parameters relative to the 5HT/NMDA control in the L2 root recordings, but only cycle period was significantly increased relative to the dopamine condition (Figure 1). There were no significant changes in any of the parameters in the L5 root recordings (Figure 2). These results suggest that I_h primarily plays an excitatory role in locomotor regulation under hyperdopaminergic conditions, like those seen in RLS. The differential effects between roots may suggest that one is more susceptible to ZD7288's effects; however, given the large degree of variability in the L5 root data, a larger sample is need to confirm this hypothesis. Further experiments include collecting more data from L5 roots to determine if there are differential effects, as well as pharmacological approaches to confirm that D1R activation upregulates I_h via the cAMP pathway.

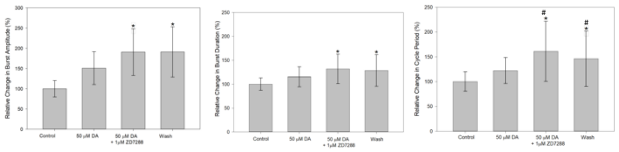

Figure 1: Mean L2 root data. The ZD7288 and wash conditions showed significant increased in all parameters relative to the 5HT/NMDA control ($p < 0.05$; represented by *), but only the cycle period was significantly increased relative to the DA condition ($p < 0.05$; represented by #).

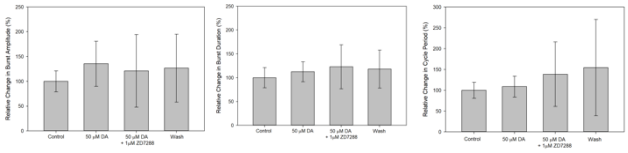

Figure 2: Mean L5 root data. There were no significant differences between any conditions in any parameters.

Faculty Mentor: Dr. Manuel Diaz-Rios
Funded by the Student Faculty Research Grant Fellowship

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