



The
UNIVERSITY
of **VERMONT**

DEPARTMENT OF ANATOMY AND NEUROBIOLOGY

GRADUATE COLLEGE DEFENSE NOTICE

Anatomy and Neurobiology Graduate Program

The following dissertation seminar is open to those in the University community.

Megan Doczi

Advisors: Deborah Damon, PhD and Anthony Morielli, PhD

"Subcellular distribution of a voltage-gated potassium channel: The effect of localization on channel function."

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12:30 PM

Davis Auditorium, FAHC/MedEd

ABSTRACT

Voltage-gated potassium channels are primary determinants of cellular excitability in the mammalian nervous system. The localization of these channels to distinct cellular compartments influences components of neuronal function, including resting membrane potential, action potential characteristics and neurotransmitter release. Thus, understanding the mechanistic basis of ion channel localization can provide fundamental insight into human physiology. The overall goal of this dissertation was to elucidate the regulatory mechanisms governing localization and function of the Kv1.3 voltage-gated potassium channel.

The sympathetic branch of the autonomic nervous system innervates many organ systems including the kidneys, heart and blood vessels and was used as a representative model to study endogenous Kv1.3. We found that postganglionic sympathetic neurons express Kv1.3 and that the channel exhibits a striking pattern of localization to the Golgi apparatus in the soma of these cells. Kv1.3 ionic current was also isolated from the soma of these neurons, indicating the channel is a determinant of the electrophysiological properties of sympathetic neurons. In addition, the specific inhibition of Kv1.3 with margatoxin was found to depolarize neuronal resting membrane potential, decrease the latency to action potential firing and increase nicotinic agonist-induced neurotransmitter release. Collectively, these findings demonstrate that Kv1.3 influences the function of postganglionic sympathetic neurons and led to the hypothesis that regulating channel localization may be a mechanism for modulating the activity of these cells.

In this dissertation, we propose that the observed Golgi retention of Kv1.3 may be a trafficking-dependent mechanism of channel regulation. To test this hypothesis, we used HEK293 cells as our model system. Our data show that the degree of Kv1.3 Golgi localization is inversely correlated with the amount of channel at the plasma membrane. In addition, the amplitude of Kv1.3 ionic current measured in cells with low Kv1.3 Golgi localization was significantly greater than the current measured in cells with high Kv1.3 Golgi localization. One mechanism for localizing ion channels to the Golgi apparatus involves the Class I PDZ-binding motif (X-S/T-X- Φ). Deletion of the C-terminal PDZ-binding motif of Kv1.3 decreased the intracellular Golgi localization of the channel and increased channel localization at the cell surface. Disrupting this canonical binding motif also increased the amplitude of Kv1.3 ionic current. These findings indicate that regulated subcellular distribution of the channel may be a determinant of Kv1.3 surface expression and function.