Second International Calcium Channel Conference - Placencia, Belize
March 28 – April 2, 2010

Characterization of the gating brake in the I-II loop of Ca_V3 T-type Ca^{2+} channels

Edward Perez-Reyes[1], Iuliia Vitko[1], Joel P. Baumgart[1], Imilla I. Arias-Olguín[2], Maria Karmazinova[3], L'ubica Lacinová[3], Sylaine Huc[4], Arnaud Monteil[4], Alexandre Mezghrani[4], and Philippe Lory[4]

[1]*Department of Pharmacology, University of Virginia, Charlottesville, Virginia , U.S.A.*
[2]*Department of Physiology, Universidad Nacional Autónoma de México, México D.F., México*
[3]*Institute of Molecular Physiology and Genetics, Slovak Academy of Sciences, Bratislava, Slovakia*
[4]*Institut de Génomique Fonctionnelle, CNRS-INSERM-Université Montpellier I & II, Montpellier, France*

Our interest was drawn to the I-II loop of $Ca_v3.2$ channels because mutations found in childhood absence epilepsy patients increased surface expression of channels, and in some cases altered their biophysical properties. To determine the roles of this loop we have studied the structure of the loop and the biophysical consequences of altering its structure. Deletions localized the gating brake to the first 55 amino acids after IS6 in all three Ca_v3 channels, establishing the evolutionary conservation of this region and its function. Circular dichroism was performed on a purified fragment of the I-II loop from $Ca_v3.2$ to reveal a high α-helical content. *De novo* computer modeling predicted the gating brake formed a helix-loop-helix structure. This model was tested by replacing the helical regions with poly-proline-glycine (PGPGPG), which introduces kinks and flexibility. These mutations had profound effects on channel gating, shifting both steady-state activation and inactivation curves, as well as accelerating channel kinetics. Mutations designed to preserve the helical structure (poly-alanine, which forms α-helices) had more modest effects. Recent gating current measurements indicate that removal of the brake shifts the *G(V)* curve to more negative potentials. Interestingly, expression of the isolated brake acts as a selective Ca_v3 dominant negative inhibitor, blocking both ionic and gating currents. Taken together, we conclude the gating brake interacts directly with S4-S5 voltage sensor hinge to stabilize it and the channel in the rested state, and this interaction is disrupted by depolarization, allowing the S6 inner bundles to open.