extracellular and intracellular mouth of the channel. This movement is critical in determining the allosteric coupling between N-type and C-type inactivation. X-ray crystallography has provided insights into the structure of low-energy stable states in inactivation. In order to investigate the path connecting the two stable end states and the sequence of movement over the energy landscape of the critical domains involved in C-type inactivation of Kv1.4 channel, we applied Φ value analysis to this channel. We chose the V561 at intracellular side of S6. Mutations:[V561A], [V561C], [V561Q], [V561S] and [V561T] were made in N-terminal deleted and N-terminal intact channels. In the N-terminal deleted constructs a Φ of 0.49 was observed for C-type inactivation. With N-terminal binding, the inactivation process became more complex, but analysis of kinetic components revealed a Φ value of 0.88 for N-terminal binding, indicating an early interaction with the open pore and a Φ value of 0.64 was obtained C-type inactivation, indicating facilitating migration of this step to earlier in the total process. Extracellular function was proved by titration of H508. The slopes of Hamond energy plot for pH titration of H508 from all different mutants are consistent with an average Φ value of 0.218 (range from 0.20 to 0.23), which indicates the movement of extracellular region near selectivity filter is after the movement of intracellular side of S6. Our results reveal that C-type inactivation can be accelerated by binding of N-terminal to the intracellular mouth of the pore, followed by conformational change at intracellular portion of S6 which is transduced to the extracellular mouth region through an allosteric mechanism.

3747-Pos Board B473
Modeling Excitability in Mechanosensory Neurons with MS Cation and MS Kv Channels
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In mechanosensory neurons, depolarizing receptor potentials arise from mechanosensitive (MS) cation channels (ECat~mV), but new findings in touch and pain neurons indicate that firing characteristics are precisely modulated by MS Kv channels. Depending on the context in which it is elicited, mechanosensory Kv activation puts the brakes on mechanosensory firing thresholds or frequencies (Hao et al 2013 Neuron 77:899). This can, for instance, ensure that only intense (noxious) mechanical stimuli generate action potential (AP) traffic in high-threshold neurons. Here we use AP modeling to explore qualitatively how Kv channel mechanosensitivity could affect threshold behaviors and AP frequency characteristics. An important unsettled question is which transition(s) in the Kv channel’s activation path accounts for Kv mechanosensitivity. Mechanosensitivity could reside with a voltage dependent step or with the thermal opening transition (Tabarean & Morris 2002 Biophys J 82:2982; Schmidt et al 2012 PNAS 109:10352). During membrane deformations this mechanistic “detail” would be consequential, we argue, because the MS transition’s location can powerfully affect the magnitude of gmax(apparent) and deactivation time course rates. To allow computational access to the specific Kv transitions, we run a hybrid HH/Markov AP model. While the standard excitability machinery is given in HH terms, the MS conductances are described in Markov fashion. To mimic the effect of a membrane stretch deformation on the MS gK, the forward/backward rates of either the concerted voltage-dependent closed-closed transition or the thermal open-closed transition are increased/decreased reciprocally (e.g., doubled/halved). Mechanosensor neurons can exhibit phasic responsiveness to sustained mechanical stimuli; to explore such behavior, terms are included that allow for the possibility that the two MS conductances, MS gCation and the MS gK, experience deformation onset/offset intensities differentially. Supported by NSERC Canada & by OHRI.

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Kcnq2 Mutation Associated with Autism and Epilepsy Impairs Inactivation Gating in Kv4.2 K⁺ Channels
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Kv4 subunits underlie somatodendritic A-type K⁺ currents in neurons. A de novo Kv4.2 mutation, V404M, was identified by whole exome sequencing in a family with identical twins affected by autism and intractable seizures. V404M immediately follows the S6 PVP motif, which is involved in activation and inactivation gating. The effects of V404M on channel function were characterized in oocytes with or without KChIP3a and DPP10a, which associate with Kv4.2 in vivo. V404M currents reached peak amplitude more slowly and decayed more slowly and less completely than wild-type currents. Because the time to peak is determined by the rates of opening and inactivation, a longer time to peak likely reflects slower inactivation. The twins’ genomes contain one wild-type and one mutant allele. To characterize channels containing both subunit types, wild type and V404M were co-expressed at a 1:1 ratio. Wild type/V404M currents resembled V404M alone indicating that the functional effects of V404M are dominant. When V404M was co-expressed with KChIP3a, little inactivation occurred during a 1 s pulse. Because KChIP3a prevents or inhibits open-state N-type inactivation, these results indicate that V404M significantly impairs closed-state inactivation. Co-expression with DPP10a confers fast open-state inactivation. However, slower closed-state inactivation was evident in V404M channels in the presence of DPP10a, indicating that V404M does not interfere with open-state inactivation conferred by DPP10a and that DPP10a does not mask the effect of V404M on closed-state inactivation. We conclude that the V404M mutation dramatically slows closed-state inactivation. In Kv4 channels, closed state inactivation likely results from uncoupling between the voltage sensor and the pore gate. We propose that replacing V404 with the larger methionine residue strengthens the electrical interaction between the gate and voltage sensor so that opening is prefered over closed-state inactivation.