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MEETING ABSTRACT

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Dual role of $Ca_V1.1$ voltage-sensing domain I in determining kinetics and voltage dependence of calcium channel activation

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Background: Voltage-gated calcium channels (Ca_V) consist of four homologous but non-identical repeats (I, II, III, IV), each containing a separate voltage-sensing domain (VSD) arranged around the common channel pore. Within each VSD the positive gating charges (K0, R1, R2, R3, R4) in the transmembrane helix S4 sequentially interact with negative counter-charges in helices S2 and S3 to support the movement of the gating charges across the electrical field of the membrane and thus to activate or deactivate the channel. Changes in VSD-I typically result in an acceleration of the characteristically slow activation kinetics of $Ca_V1.1$, while VSD-IV acts as critical determinant of the voltage-dependence of activation. Recently, we showed that a conservative amino acid substitution (R165K) of an outer gating charge (R1) in VSD-I resulted in a right-shifted voltage dependence of activation, without changing its kinetics. This indicated a cooperativity of VSDs I and IV in determining the voltage dependence of $Ca_V1.1$ channel gating. Here, we examined the molecular mechanism by which VSD-I of $Ca_V1.1$ controls voltage sensitivity and kinetics of channel activation.
Methods: Structure modeling of $Ca_V1.1$ predicted putative ion-pair interaction partners of VSD-I gating charges with negative counter-charges in S2. The functional relevance of these residues was tested experimentally using site-directed mutagenesis and whole-cell patch clamp analysis of channels reconstituted in dysgenic myotubes.
Results: Two glutamates at the extracellular end of the IS2 transmembrane helix were predicted to form ion pairs with the gating charges R3, R2, and R1 sequentially during the activation process. Mutating both of these residues (E87A, E90A) resulted in a striking acceleration of the activation kinetics as well as in a 17 mV right-shift of the voltage dependence of activation ($V_{½}$). Similar effects on $V_{½}$ and kinetics were obtained when mutating only E90A. However, the single mutation of E87A only shifted $V_{½}$ without accelerating kinetics of activation.
Discussion: These results demonstrate a twofold role of VSD-I in regulating voltage dependence and kinetics of activations. Together with molecular dynamics simulation of $Ca_V1.1$ in resting and activated states (see accompanying poster by Monica Fernández Quintero [1]) the mutagenesis study suggests that ion-pair interactions stabilizing the activated state increase the voltage sensitivity of the VSD, whereas ion-pair interactions stabilizing resting states slow down the voltage-sensor movement and thus contribute to slow activation of $Ca_V1.1$ calcium currents.
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Reference

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