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MEETING ABSTRACT

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Structure modeling of $Ca_V1.1$ calcium channels reveals functional inter- and intradomain interactions involved in voltage sensing

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Background: Voltage-gated calcium channels (CaV) 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 (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. Previously we identified an interaction of R1 and R2 with aspartate 1196 (D4) in S3 of the VSD-IV of $Ca_V1.1$ that is critical for modulation of voltage sensitivity and current density by alternative splicing in the IVS3–S4 linker.

Methods: The structure of $Ca_V1.1$ [1] was modeled in a lipid environment and enhanced molecular dynamics (MD) simulations of VSDs I and IV were performed to identify resting states. Free energy maps and transition times were calculated using a Markov state model. Residues predicted to function as counter-charges in the activation/deactivation process were examined using site-directed mutagenesis and voltage-clamp analysis.

Results and discussion: An interaction with a glutamate (E216) in S5 of VSD-I that participates in the R1 / R2–D4 interaction of VSD-IV was identified by structure modeling. Charge neutralization (E216Q) or substitution with alanine (E216A) in $Ca_V1.1e$ caused a 7 mV and 16 mV, respectively, right-shift of voltage dependence of activation and a 30% reduction in current density. This effect is specific to the splice variant lacking exon 29 and is quantitatively in the same range as previously observed when R1, R2, or D4 were mutated. This finding indicates that a trans-domain interaction between repeats I and IV participates in the voltage-dependent gating and its modulation by alternative splicing. Furthermore, structure models of $Ca_V1.1$ in the activated and resting states show that the four VSD differ greatly regarding their intra-domain interactions, consistent with their specific contributions to $Ca_V1.1$ gating properties. In particular, we found that the gating charges (R1, R2, R3) of VSD-I form multiple sequential interactions with counter charges in the S2 helix. MD simulation indicated that these interactions stabilize S4 also in its resting states, and thus endow VSD-I with thermodynamics properties corresponding to its slow activation kinetics. These predictions were confirmed experimentally (see accompanying poster by Yousra El Ghaleb [2]) and allow devising the first mechanistic model to explain the current properties of a voltage-gated ion channel based on the molecular properties of its structurally and functionally distinct VSDs.

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Keywords: $Ca_V1.1$ channels – voltage gating – activation kinetics – voltage sensor – homology modeling – molecular dynamics simularions – Markov state models

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