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Meeting abstract

Second-hit kinetic perturbations reveal structural features of the domain IV S6 segment associated with fast inactivation

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Background

In the voltage-gated Na^+ channel the central pore is believed to be lined by the S6 segments of all four domains. Conformational changes of these S6 segments are thought to give rise to channel opening, closing and fast inactivation (FI).

Methods

In order to reveal structural features of the domain IV S6 segment (DIV-S6) relevant to FI, we produced serial replacements by cysteines of residues 1575–1591 in DIV-S6 of the $rNa_V1.4$ channel ("first hit"). Furthermore, a critical residue within the selectivity filter of the $rNa_V1.4$ channel, K1237, was replaced by the negatively charged glutamate ("second hit"). Previously, this mutation has been shown to produce dramatic alterations in permeation and gating properties of the wild type channel. The constructs were expressed in *Xenopus laevis* oocytes and studied by two electrode voltage clamp.

Results

In the background of serial cysteine replacements in DIV-S6, the mutation K1237E shifted the half-point of FI to more negative potentials in all but two constructs. Only in the DIV-S6 background of F1579C and Y1586C was the half-point of FI shifted to more positive potentials upon addition of K1237E. This is remarkable, because both residues have previously been implicated in destabilization of FI and in binding of local anesthetics to the channel. A fast Fourier analysis of the serial "second hit" changes in the voltage-*independent* free energy of FI revealed a periodicty of gating perturbations by K1237E consistent with an alpha-helical arrangement of DIV-S6. Such periodicity could not be found if the voltage-*dependent* charge movement associated with FI-perturbations by K1237E was analyzed.

Conclusion

We conclude that residues F1579 and Y1586 in DIV-S6 serve to stabilize FI. Furthermore, the movement of the inactivation gate during FI appears to be associated with an alpha-helical arrangement of DIV-S6.

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