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Double mutant gating perturbation analysis predicts a high conformational stability of the domain IV S6 segment of the voltage-gated Na^+ channel

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Background: The S6 segment of domain IV (DIV-S6) of voltage-gated Na^+ channels is considered to be a key player in gating and local anesthetic drug block. Thus, mutations at several sites of DIV-S6 are known to substantially alter the channel's inactivation properties.

Methods: For a comprehensive analysis of the kinetic role of DIV-S6 in fast inactivation we performed a cysteine scanning analysis of sites 1575-1591 in the DIV-S6 of the $rNa_V1.4$ channel. These mutations were engineered into the wild-type channel and into $rNa_V1.4$ carrying the mutation K1237E. K1237 is located in the P-loop of domain III and mutations at this site have dramatic effects both on permeation and gating properties. Hence, K1237E most likely causes a complex conformational change of the channel. We sought to explore whether K1237E changes the pattern of gating perturbations produced by the serial cysteine replacements in DIV-S6. The constructs were expressed in *Xenopus laevis* oocytes and studied by means of two electrode voltage-clamp.

Results: The half-point of availability following a 50 ms conditioning prepulse (V05) was −44 ± 1 mV and −51 ± 1 mV in wild-type and K1237E, respectively ($p < 0.001$). Most serial amino acid replacements by cysteines in DIV-S6 produced shifts in V05, both in the background of wild-type and in the background of K1237E, ranging from +17 ± 1 mV to −9 ± 2 mV. A plot of the shifts in V05 by single DIV-S6 mutants relative to wild-type vs. the shifts in V05 by double mutants relative to K1237E showed a significant positive correlation ($r = 0.92$, $p = 0.002$).

Conclusions: This indicates that the general pattern of gating perturbations in DIV-S6 is not affected by K1237E, suggesting a high conformational stability of the DIV-S6 segment during the fast inactivated state.

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