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INTRINSIC ACTIVITY

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MEETING ABSTRACT

A1.4

The unique characteristics of segment IS4 in voltage-gated calcium channel $Ca_V1.2$ inactivation

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Background: Voltage-dependent inactivation of the voltage-gated calcium channel $Ca_V1.2$ develops during the plateau phase of the cardiac action potential and enables timed repolarization and tuned calcium entry. Recently solved structures of $Ca_V1.1$ provided the first models for interpretation of opening/closing behavior in the Ca_V1 family on a structural basis. However, the molecular events during $Ca_V1.2$ inactivation remain unclear. There exists a correlation between the positions of the steady-state activation and inactivation curves in this channel; this may reflect two scenarios: either conformational changes in the pore trigger voltage-dependent inactivation by allosterically changing to the selectivity filter, or, both processes are coupled via conformational changes in the voltage-sensing domains (VSDs).

Methods: In order to investigate the above given hypotheses, we used $Ca_V1.2$ constructs with fully or partially neutralized charges in S4 segments to elucidate the role of VSD I–IV in voltage-dependent inactivation. Charged arginines or lysines were replaced by glutamines in down-stream direction using site-directed mutagenesis. Ionic currents were recorded using patch-clamp measurements. Furthermore, homology models of all four voltage-sensing domains were generated.

Results: (i) Compared to the lower impacts of IIS4 and IIIS4, neutralization of IS4 charges induced pronounced changes in voltage-dependence and kinetics of inactivation. (ii) Neutralization of IS4 charges gradually reduced the slope factors of the inactivation curves and accelerated the inactivation kinetics. (iii) Shifts of the inactivation curves induced by IS4 neutralization correlated with shifts of the activation curve.

Discussion: Bezanilla and Villalba-Galea [1] show that to estimate the effective charge using a Boltzmann fit is not precise, if the S4 movement occurs in multiple steps via sub-states. Given this assumption, the distribution between inactivated and open channels would be shallower than predicted by a two-state Boltzmann function. It is thus tempting to speculate that IS4 in $Ca_V1.2$ channel moves via sub-states that are themselves stabilized by interactions of arginines (and lysines) with their surrounding environment. Replacing these charged residues by neutral glutamines would consequently disrupt these interactions, thereby reducing the number of IS4 sub-states and decreasing the slope factor of the inactivation curve.

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Reference

1. Bezanilla F, Villalba-Galea CA: **The gating charge should not be estimated by fitting a two-state model to a *Q-V* curve.** *J Gen Physiol,* 2013; 142(6):575–578. doi:10.1085/jgp.201311056

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