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MEETING ABSTRACT

A4.8

Neutralization of the voltage sensor in domain I strongly affects activation and inactivation gating in $Ca_V1.2$

Stanislav ANDRANOVITŠ, Stanislav BEYL, Annette HOHAUS, Eugen TIMIN and Steffen HERING*

Department of Pharmacology and Toxicology, University of Vienna, Austria

Background: Voltage-gated calcium influx through $Ca_V1.2$ regulates numerous cellular functions. These channels have four S4 segments in domains I–IV carrying between 5 and 6 charges, respectively. Here, we ask whether IS4–IVS4 differentially affect activation and inactivation of $Ca_V1.2$.

Methods: To probe their role in $Ca_V1.2$ gating processes we partially or completely replaced arginines and/or lysines in IS4–IVS4 by glutamines. HEK 293 cells were co-transfected with cDNAs encoding wild-type or mutant $Ca_V1.2$ α1 subunits with auxiliary β3a as well as α2–δ1 subunits. Currents were measured in "high barium" (20 mM) extracellular solution. Activation/inactivation of barium inward currents was analyzed using the patch clamp technique.

Results: Charge-neutralising point mutations in S4 segments shift the activation and inactivation curves by similar amounts on the voltage axis. Regression analysis of half-activation voltage *vs.* half-inactivation potential exhibits significant correlation ($r = 0.95$, $p < 0.015$). Substitution of charged residues in IS4 induced the strongest shifts of the activation/inactivation curves. $Ca_V1.2$ carrying only one charge in lower position (R4) opens at more negative potentials. Increasing the number of charged residues until four gradually moved the activation curve towards more depolarized voltages. Compared to domain I, mutations in domain II did not substantially affect the voltage dependence. Neutralization of the upper charge in IVS4 (R1: R1359Q) shifted the activation and inactivation curves like the IS4N+4 (K264Q) mutant (equal right shift of about 13.5 mV) while double mutants (***e.g.*** R1359Q/R1362Q) failed to form functional channels.

Discussion: Our data support the hypothesis that activation and inactivation in $Ca_V1.2$ are coupled processes. We hypothesise also that IS4 strongly stabilizes the closed and inactivated states, suggesting a key role of this segment in $Ca_V1.2$ activation and inactivation gating.

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*Corresponding author e-mail: *steffen.hering@univie.ac.at*