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Exploring biophysical and pharmacological properties of Nav1.5 channels with experimental and simulation methods

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We performed voltage-clamp experiments on outside-out patches excised from HEK293 cells stably expressing hNav1.5. Upon 3-min exposure to a 808.5 nm laser the peak Na+ current amplitude remained steady (96.64±5.25% of initial values) while in control conditions it decreased (37.95±9.14%); in whole-cell experiments relative amplitudes were 111.2±47.0% (laser) vs. 70.6±33.0% (control). We also titrated the effects of cenobamate on hNav1.5 peak and late current (with ATX-II 100 nM in bath) and found apparent IC50 of 87.6 μM (peak) and 46.5 μM (late). Using a multi-pulse voltage-clamp protocol to assess use-dependent block and recurrence formulae for peak current amplitudes at consecutive pulses obtained with supplementary simplifying assumptions, we estimated state-specific blocking/unblocking rates: for open-state kob=0.00215μM-1ms-1, kob-1=0.189ms-1, for inactivated-state kib=0.0006698μM-1ms-1, kib-1=0.18252ms-1. Applying these and other in vitro pharmacology data (IC50 for hCav1.2, hKv7.1, hERG) to a modified O'Hara-Rudy2011 human ventricular cardiomyocyte electrophysiology model in a 1D string of 50 cardiomyocytes with different connectivities, we found out that at 20-fold reduced intermyocyte gap-junction conductivity (300pS/pF) cenobamate produced significant conduction velocity decreases (0.0148m/s at 0.5 x Cmax vs. 0.0259m/s-control). We obtained an open-conformation 3D model of hNav1.5 using AlphaFold2, we embedded it in a DPPC bilayer model with CHARMM-GUI, corrected residue protonation state (pH7.2) with H++, added 2 Na+ ions in the selectivity filter. By molecular docking we found the cenobamate binding site in the central cavity. We identified 10 mutant variants in the binding site region and explored them via docking and MD followed by MM-PBSA: mutants N1463K/Y and M1766R feature higher predicted inhibitory affinity than wild-type channels.
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