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MEETING ABSTRACT

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Mutation-induced changes in channel gating parameters increase sensitivity of pathogenic $Ca_V1.3$ L-type Ca^{2+} channel variants towards the Ca^{2+} channel blocker isradipine

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Background: Pathogenic *de novo* missense variants in the pore-forming α1 subunit (*CACNA1D*) of $Ca_V1.3$ voltage-gated Ca^{2+} channels cause a neurodevelopmental disorder with or without endocrine symptoms. We have shown that these variants enhance channel function and that some of them display higher sensitivity for the dihydropyridine (DHP) L-type Ca^{2+} channel blocker isradipine. Currently licensed DHPs may therefore provide a potential off-label treatment option in affected patients. To elucidate the mechanism leading to the higher isradipine sensitivity of disease variants, we tested if this can be solely explained by the enhanced voltage-dependent inactivation introduced by these variants as predicted by the modulated receptor hypothesis (MRH) [1].

Methods: We expressed wild-type (WT) $Ca_V1.3$ as well as variants A749T and L271H together with β2a and α2-δ1 subunits in HEK 293T cells and measured isradipine sensitivity using 50-ms pulses (0.1 Hz) from various holding potentials (HP; −89 mV to −27 mV) to vary the fraction of inactivated channels.

Results: Both A749T ($V_{0.5,act}$: −35.3 mV) and L271H ($V_{0.5,act}$: −41.7 mV) variants caused significant shifts in steady-state inactivation (SSI) towards negative potentials compared to WT ($V_{0.5,act}$: −16.5 mV). At −89 mV HP, A749T showed 1.4-fold higher sensitivity to isradipine (IC_{50}: 93.3 nM) compared to WT (IC_{50}: 134.9 nM) and 2.4-fold higher potency at −54 mV HP (IC_{50}: 17.3 nM) compared to WT (IC_{50}: 42.9 nM). This is consistent with voltage-dependent inhibition favored by a higher degree of inactivation of A749T channels at a given HP compared to WT. Accordingly, at HPs selected to stabilize similar SSI of 10 % and 20 %, respectively, for WT and A749T, IC_{50} values were no longer significantly different between A749T ($IC_{50(10\%)}$: 7.00 nM; $IC_{50(20\%)}$: 3.27 nM) and WT ($IC_{50(10\%)}$: 5.50 nM; $IC_{50(20\%)}$: 3.24 nM). These data suggest that enhanced voltage-dependent inactivation alone can explain the increase in isradipine sensitivity of this variant. We also observed a voltage-dependent five-fold increase of the isradipine sensitivity for variant L271H in comparison to WT at −89 mV HP but with a significantly lower IC_{50} at a predicted 10 % SSI ($IC_{50(10\%)}$: 1.80 nM) compared to WT and A749T.

Discussion: WT, A749T and L271H channels are inhibited by isradipine in a strongly voltage-dependent manner. SSI at more negative voltages of the A749T variant accounts for its higher isradipine sensitivity, because more inactivation occurs at a given voltage compared to WT. The voltage-dependent increase of the isradipine sensitivity of WT and A749T channels can be quantitatively predicted from the modulated receptor hypothesis (MRH) by assuming affinities of 134.9 nM for resting and 0.70 nM for inactivated channels. We currently investigate whether the higher sensitivity of variant L271H to isradipine at depolarized voltages is explained by its larger inactivation during our recording protocol. Our data encourage ongoing efforts for the symptomatic treatment of affected individuals with isradipine.

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