1617-Pos

Mutation of Cardiac Nav1.5 in an Hisian-Type Arrhythmia, Associated with Dilated Cardiomayopathy

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Cardiac sodium channels are complexes including alpha and beta1 subunits allowing sodium influx during the depolarization phase of the ventricular action potential. The pore-forming alpha subunit, Nav1.5, is encoded by *SCN5A*. Using a candidate-gene approach, we detected a variant of *SCN5A*, leading to the R222Q substitution by screening one family with cardiac arrhythmia resulting in frequent premature ventricular contractions, non sustained ventricular tachycardia and dilated cardiomyopathy. Arrhythmia mechanisms involved ectopic foci originating from the proximal part of the His-Purkinje system. To evaluate the incidence of this substitution on Nav1.5 function, whole-cell patch-clamp experiments were performed on COS-7 cells transfected with the human alpha and beta1 subunits. The presence of the mutation at the heterozygous or homozygous state did not modify the sodium current density. In contrast, the activation curve was shifted toward more negative potentials (V1/2act, WT: -30.6 ± 2.1 mV, n=11; R222Q: -42.3 ± 1 mV, n=11, $p<0.001$; heterozygous: -37.2 ± 1.6 mV, n=9; $p<0.05$) and the slope was changed in the heterozygous condition only (WT: 5.7 ± 0.3 mV, R222Q: 6.5 ± 0.4 mV, heterozygous: 7.1 ± 0.3 mV; $p<0.01$). Activation kinetics were also accelerated in mutant homozygous condition only ($p<0.001$, versus WT). Inactivation voltage sensitivity was also changed (V1/2inact, WT: -79.6 ± 0.7 mV, n=10; R222Q: -84.6 ± 0.7 mV, n=8, $p<0.001$; heterozygous: -82.2 ± 1 mV, n=9; $p<0.05$), its kinetics accelerated ($p<0.001$ versus WT for both mutant and heterozygous conditions) and the slope was changed in the mutant homozygous condition only (WT: 5.6 ± 0.2 mV; R222Q: 4.8 ± 0.2 mV; $p<0.01$; heterozygous: 5.3 ± 0.1 mV). Finally, recovery from inactivation was not modified by the R222Q mutation. We studied the impact of the current biophysical changes in cellular models of the Purkinje and ventricular action potentials. The premature ventricular contractions are explained by the appearance of electrical abnormalities rather in Purkinje fibers than in ventricular cardiomyocytes.

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Block and Permeation of the Hypokalemic Periodic Paralysis Gating Pore In Nav1.4 Channels

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We reported previously that naturally occurring hypokalemic periodic paralysis (HypoPP) mutations of voltage sensing arginines in domain II of the skeletal muscle sodium channel $Na_v1.4$ produce gating pore currents at hyperpolarized membrane potentials. These small but persistent currents produce a gain-of-function that would contribute to the pathophysiology of HypoPP. Here we investigate biophysical properties of the gating pore with mutations in R2 in more detail. We confirm that Na^+ currents through the gating pore can be blocked by Ba^{2+} and Zn^{2+} at mM concentrations. Block is voltage-dependent and is substantially increased by strongly negative holding potentials. Voltage-dependent block develops with kinetics consistent with preferential binding of divalent cations to the resting conformation of the voltage sensor. Trivalent cations such as Gd^{3+}, La^{3+}, Yb^{3+} block Na^+ gating pore currents with higher affinity than divalents (hundreds of nM), but with much less voltage dependence. We also probed permeation through the gating pore. Currents through the $Na_v1.4$/R2G gating pore carried by guanidinium (Gu^+) are ~25 fold larger than Na^+ currents. Smaller derivatives like ethyl-guanidine also permeate through the gating pore better than sodium. Bulkier guanidine derivatives block both Na^+ and Gu^+ gating pore conductances at mM concentrations. Interestingly, HypoPP mutant $Na_v1.4$/R2H, which is proton-selective in physiological saline, is also permeable to Gu^+ despite its lack of permeability to monovalent alkali metal cations. The high Gu^+ permeation through these gating pores is consistent with the expected favorable environment for the guanidinium side chains of the native arginine gating charges. Our studies reveal conformation-dependent divalent cation block of these HypoPP mutant gating pores, as well as block by guanidine derivatives, which may provide potential targets for therapeutically active compounds.

1619-Pos

Mutation in Nav1.5 Associated with Brugada Syndrome - a Mutational Hotspot?

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Several studies have demonstrated an association between Brugada syndrome (BrS) and mutations in genes encoding ion channel subunits including SCN5A, CACNA1C, CACNB2b, SCN1B, and KCNE3. Mutations in SCN5A, encoding the voltage-gated sodium channel Nav1.5, represent the majority with greater than 293 mutations in SCN5A linked to the syndrome (Kapplinger et al, Heart Rhythm, In press 2009). We identified a missense mutation (G1408R) in SCN5A in a large BrS family. Intriguingly, this mutation had been reported earlier in two independent studies and has also shown to be associated with Sick Sinus Syndrome (SSS) and Cardiac Conduction Defect (CCD) (Kyndt et al, 2001; Benson et al, 2003). Nav1.5-G1408R channels heterologously expressed in CHO cells and studied using patch-clamp techniques failed to generate any sodium channel current (INa). Co-expression of the mutated channels with wild-type (WT) channels resulted in a 50% reduction of current amplitudes with no changes in kinetic properties when compared with WT channels. The residue resides in the DIII pore region and is conserved among species. We addressed the importance of this amino acid at position 1408 by replacing it with another small neutral amino acid (G1408A) and by substituting a negatively charged aspartic acid (G1408D). Our results show that substituting glycine with alanine retains WT behavior while exchange to the positively charged arginine (G1408R) or negatively charged aspartic acid leads to a complete loss-of-function. In conclusion, we describe a SCN5A mutation associated with BrS which results in a loss of function of INa important for action potential generation. Further, we show that the presence of a neutral hydrophobic amino acid at this position is crucial for normal channel function.

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Do Sodium Channel α - α Interactions Contribute to Loss-of-Function Observed in Brugada Syndrome?

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The pathogenesis of Brugada Syndrome (BrS) has been associated with mutations in the cardiac sodium channel gene SCN5A, resulting in loss-of-function. Recently, the L325R mutation has been proposed to cause BrS through a dominant-negative effect. Dominant-negative effects are usually the consequence of mutant subunits assembling with wild-type (WT) into non-functional channel multimers. In contrast, sodium channel α-subunits are not believed to oligomerize. However, there is increasing evidence suggesting the existence of α-α interactions between sodium channels. Therefore, we tested whether the dominant-negative effect seen in some BrS mutations is due to interactions between sodium channel α-subunits. We co-expressed a dominant-negative BrS mutation, L325R, with WT channels at different molar ratios. Channels containing the mutation alone did not elicit current. When WT and L325R channels were co-transfected in a 1:1 and 4:1 WT:L325R ratios, the normalized peak INa densities were reduced respectively to $29.8 \pm 6.2\%$ and $57.3 \pm 5.8\%$ of the control WT confirming the dominant-negative effect of this mutation. When using a binomial distribution, our results were fitted best by a configuration suggesting the interaction of two channel monomers. We also investigated the existence of channel-channel interactions using the BrS mutation L567Q. This mutation displays biophysical alterations possibly too small to explain the clinical phenotype. Interestingly, co-expression of L567Q with WT channels produced a significant reduction in INa density which could possibly also be caused by channel-channel interactions and therefore explain the clinical manifestation of the disease. In conclusion, our experiments using BrS mutations, now suggest the idea of a dimerization of sodium channel α-subunits.

1621-Pos

An Intronic Mutation of SCN4A Associated with Myotonia Raises an Aberrantly Spliced Isoform with Disrupted Fast Inactivation

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