**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: $-30.6 \pm 2.1$ mV, n=11; R222Q: $-42.3 \pm 1$ mV, n=11, $p<0.001$; heterozygous: $-37.2 \pm 1.6$ mV, n=9; $p<0.05$) and the slope was changed in the heterozygous condition only (WT: $5.7 \pm 0.3$ mV, R222Q: $6.5 \pm 0.4$ mV, heterozygous: $7.1 \pm 0.3$ 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: $-79.6 \pm 0.7$ mV, n=10; R222Q: $-84.6 \pm 0.7$ mV, n=8, $p<0.001$; heterozygous: $-82.2 \pm 1$ 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: $5.6 \pm 0.2$ mV; R222Q: $4.8 \pm 0.2$ mV; $p<0.01$; heterozygous: $5.3 \pm 0.1$ 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.

**1618-Pos**

**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.

**1620-Pos**

**Do Sodium Channel $\alpha$ - $\alpha$ 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 $\alpha$-subunits are not believed to oligomerize. However, there is increasing evidence suggesting the existence of $\alpha$-$\alpha$ interactions between sodium channels. Therefore, we tested whether the dominant-negative effect seen in some BrS mutations is due to interactions between sodium channel $\alpha$-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 $\alpha$-subunits.

**1621-Pos**

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

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Genetic defects of voltage-gated ion channel genes are responsible for several inheritable skeletal muscle diseases. Mutations of skeletal muscle sodium channel, SCN4A, have been shown to associate with myotonia and periodic paralysis. So far, most of the disease mutations of SCN4A are located in coding regions and the mutated channels show disruption of fast inactivation or enhancement of activation. We found a patient with myotonia caused by aberrantly spliced channel due to a mutation located at an intron of SCN4A. Moreover, this mutant channel showed gain of function defect, disruption of fast inactivation.

A case was 35 year-old male who showed well-developed Herculean musculature and generalized muscle stiffness aggravated with cold exposure. We performed sequence analysis of SCN4A and CLCN1 using genomic DNA extracted from patient's lymphocytes. Moreover the mRNAs of SCN4A expressed in patient's muscle were analyzed by RT-PCR and nucleotide sequence. We constructed an expression vector of the channel isoform expressed in patient's muscle and measured Na current with whole cell configuration using HEK293t cells on which channels are expressed transiently.

No mutations were identified in all exons of either SCN4A or CLCN1. Replacement of 5 nucleotides to a single nucleotide was detected in intron 21 of SCN4A. The mutation is located at downstream of exon 21 which could serve as splicing donor site. RT-PCR and nucleotide sequence analysis of cDNA showed three aberrantly spliced isoforms. The only in-frame isoform should result in insertion of 35 amino acids between domain III and IV. This isoform expressed in HEK cells showed marked defect in fast inactivation.

Disease mutations located at non-coding region of voltage-gated ion channel genes usually show loss-of function, and this is the first example which shows gain-of function defect.

**1622-Pos**

**Properties of the Domain-II Voltage Sensor Determining the Function of $Na_v1.8$ (SCN10A) Channels**

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$Na_V1.8$ voltage-gated sodium channels are primarily expressed in dorsal root ganglia neurons and are implicated in pain perception. One of their remarkable features is their activation at relatively depolarized membrane voltages ($V_a = -5.3 \pm 1.5$ mV compared to $-40.2 \pm 2.2$ mV for skeletal muscle $Na_V1.4$). Searching for the underlying molecular determinants, we constructed domain chimeras between rat $Na_V1.4$ and $Na_V1.8$ channels and assayed them with the whole-cell patch-clamp method after expression in Neuro-2A cells. While we could not obtain any current response with 8444, i.e. a construct with domain-I from $Na_V1.8$ and the remaining domains from $Na_V1.4$, analysis of chimeras 4844 ($V_a = -12.8 \pm 2.2$ mV), 4484 ($-40.4 \pm 1.5$ mV) and 4448 ($-25.7 \pm 2.0$ mV) suggests that the depolarized activation voltage mainly results from domain-II. The voltage sensor in domain-II of $Na_V1.8$ harbors a double KK motif in the S3/S4 linker (KK726 and KK747), a feature unique for $Na_V1.8$ channels. Mutating the KK motifs to the corresponding residues of $Na_V1.4$ results in a left-shift in activation for KK747NV ($V_a = -15.6 \pm 1.3$ mV). The reverse mutations in $Na_V1.4$, QG634KK and NV655KK, have a qualitatively opposite effect. Furthermore, the KK motifs seem to interfere with the μO-conotoxin MrVIA, which is known to interact with the voltage-sensor of domain II via a sensor-trapping mechanism. Mutants KK747NV and KK726QG-KK747NV are blocked by 400 nM MrVIA by $91 \pm 1\%$ and $86 \pm 8\%$ respectively, compared to $64 \pm 4\%$ for $Na_V1.8$ wild-type channels. In addition, toxin dissociation at +40 mV is about 1.5 times faster for KK726QG-KK747NV than for wild-type channels. Thus, the KK motifs in the S3/S4 linker of $Na_V1.8$ domain-II voltage-sensor take part in channel gating compatible with the ''paddle model'' and provide a molecular explanation for the gating mechanism unique to $Na_V1.8$ channels.

**1623-Pos**

**A Conserved Double-Tyrosine Motif in the Cardiac Sodium Channel Domain III-IV Linker Underlies Calcium Dependent Ca2+/Calmodulin Binding and Regulation of Inactivation Gating**

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Voltage-gated sodium channels maintain the electrical cadence and stability of neurons and muscle cells by selectively controlling the transmembrane passage of their namesake ion. The degree to which these channels contribute to cellular excitability can be managed therapeutically or fine-tuned by endogenous ligands.

Intracellular calcium, for instance, heavily modulates sodium channel inactivation, the process by which sodium conductance is negatively regulated. We explore the molecular basis for this effect by investigating the interaction between the ubiquitous calcium binding protein calmodulin (CaM) and the putative sodium channel inactivation gate comprised of the cytosolic linker between homologous channel domains III-IV (DIII-IV). Experiments using isothermal titration calorimetry (ITC) show that CaM binds in a calcium-dependent manner to a novel motif in the center of the DIII-IV linker, N-terminal to a region previously reported to be a CaM binding site. An alanine scan of aromatic residues in recombinant DIII-DIV linker peptides reveals that while multiple side-chains contribute to CaM binding, two tyrosines (Y1494 and Y1495) play a crucial role in binding the CaM C-lobe. The functional relevance of these observations is then ascertained through electrophysiological measurement of sodium channel inactivation gating in the presence and absence of calcium. Experiments on patch-clamped transfected tsA201 cells show that only the Y1494A mutation of the five sites tested renders sodium channel steady-state inactivation insensitive to cytosolic calcium.

The results demonstrate that calcium dependent CaM binding to the DIII-IV linker double tyrosine motif is required for calcium regulation of the cardiac sodium channel.

**1624-Pos**

**Extracting Thermodynamic Parameters from Site-Specific Observables: Chi-Value Analysis**

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Interactions between different structural domains are important determinants of the function and regulation of allosteric proteins. Prevalent theories to understand the thermodynamic basis of cooperativity have frequently constrained the interactions between the different domains to come up with a mathematical model which can reproduce the complex behavior of a global thermodynamic observable such as ligand binding or enzyme activity. Such simple phenomenological models, in general, are not amenable to molecular description of cooperative interactions. Here, we provide the theoretical framework of a method, the chi-value analysis, which can elucidate the thermodynamic effect of molecular level perturbations. The chi value analysis involves extracting a site-specific parameter (chi value) associated with a structural domain and observing how the chi value is altered by a mutation. Through this formalism, based on classical statistical mechanics, we show that the chi value analysis can be used to deconstruct and quantify the energetic effects of mutations even in a complex macromolecular system consisting of a large number of structural units interacting via a complex network of interactions. Using models of the multi-domain voltage-dependent sodium and large-conductance calcium activated potassium channels, we have performed extensive numerical simulations to probe the validity of the chi value analysis. Our theory may provide a general approach to understand the detailed energetics underlying cooperative behavior of multi-domain proteins.

**1625-Pos**

**Modeling the Outer Pore of Sodium and Calcium Channels**

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In the absence of x-ray structures of sodium and calcium channels, their homology models based on x-ray structures of potassium channels are used to design and rationalize experiments. A challenge is to model the outer-pore region that folds differently from potassium channels. Here we report a model of the outer-pore region of NaV1.4, which is based on a large body of experimental data, including specific contacts of toxins with individual channel residues. The model inherits from our previous model the general disposition of the P-helices, the selectivity-filter residues, and the outer carboxylates, but provides a novel view on the role of other highly conserved residues in the outer pore. In the absence of secondary-structure elements, structural stability of the outer pore should be supported by specific contacts. We propose a network of such contacts including intra- and inter-domain H-bonds, knob-into-the-hole contacts, and hydrophobic interactions. Glycine residues downstream the selectivity filter are proposed to participate in knob-into-hole contacts with P-helices and S6s. These contacts explain known tetrodotoxin resistance of snakes adapted to toxic prey due to NaV1.4 mutation Ile/Val in the P-helix of repeat 4. Polar residues in P-helices, which are five positions upstream from the selectivity-filter residues, form H-bonds with the ascending-limb backbones. The exceptionally conserved tryptophans are engaged in inter-repeat H-bonds to form a ring whose π-electrons would facilitate the passage of ions from the outer carboxylates to the selectivity filter. The outer-pore model of CaV1.2 derived from the NaV1.4 model is also stabilized by the ring of exceptionally conservative tryptophans and H-bonds between P-helices and ascending limbs. In this model a highly conserved aspartate downstream the selectivity-filter glutamate in repeat II facilitates passage of calcium ions moving to the selectivity-filter ring through the tryptophan ring.

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