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 a 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 analysis. We constructed an expression vector of the channel isoform expressed in 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 the 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 Na1.8 (SCN10A) Channels
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Na1.8 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 (VA = -5.3 ± 1.5 mV compared to -40.2 ± 2.2 mV for skeletal muscle Na1.4). Searching for the underlying molecular determinants, we constructed domain chimeras between rat Na1.4 and Na1.8 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 rat Na1.8 and the remaining domains from Na1.4, analysis of chimeras 4844 (VA = -12.8 ± 2.2 mV), 4484 (-40.4 ± 1.5 mV) and 4444 (-25.7 ± 2.0 mV) suggests that the depolarized activation voltage mainly results from domain-II. The voltage sensor in domain-II of Na1.8 harbors a double KK motif in the S3/S4 linker (KK726 and KK747), a feature unique for Na1.8 and absent in Na1.4. While the KK motif is conserved in other vertebrates, the corresponding residues of Na1.4 results in a left-shift in activation for KK747NV (VA = -15.6 ± 1.3 mV). The reverse mutations in Na1.4, 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% and 86% respectively, compared to 64 ± 4% for Na1.8 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 Na1.8 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 Na1.8, 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 the nameake 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 mathematical 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 of NaV1.4 which 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 IleVal 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 P-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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