Na channels. The aim of this study was to assess the role of Na\(^+\) channels in IQ/AA\(^+\) mice. Sodium channel mutations near the IQ and EFL motifs in the carboxyterminal domain of Nav1.5 contribute to more depolarized voltages, an effect referred as inactivation slowing. For several mutations, shifts in the steady-state inactivation curve are observed that cause an increase in current availability may be associated with increased risk for these patients. In addition, we measured mutation specific effects of ranolazine for these mutants. For all mutants tested, ranolazine preferentially blocked late sodium currents. Ranolazine shifts steady-state availability of the inactivation process was dependent on the specific mutant tested. Our results suggest that ranolazine may have a mutation dependent effect. Mutations dysfunction may affect drug binding and drug actions in the channel and may alter treatment effectiveness in patients.

**2901-Pos Board B331**

Rotational Symmetry of Two Pyrethroid Receptor Sites in the Mosquito Sodium Channel

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Pyrethroid receptor sites target voltage-gated sodium channels. Emerging mosquito resistance to widely used pyrethroids demands development of new insecticides. Earlier the X-ray structure of the open Kv1.2 channel and mutagenesis data were used to build two homology models of insect sodium channels with pyrethroid receptors PyR1 (O’Reilly et al., 2006) and PyR2 (Du et al., 2015) located, respectively, in the II/III and I/I domain interfaces. The models differ in the number of contributing transmembrane helices, orientation of the bound pyrethroid molecules, and the depth of their penetration into the hydrophobic domain interfaces. Here we employed our PyR2 model to elaborate an analogous PyR1 model. Computational docking yielded a revised PyR1 model with deltamethrin bound between the linker helix IIIS4-S5 and transmembrane helices IIIS5, IIIS6, and IIIS6 with its dibromoethenyl and diphenylether moieties oriented, respectively, in the intra- and extracellular directions. Comparison of the PyR2 and revised PyR1 models predicted new deltamethrin-channel contacts. Model-driven mutagenesis followed by electrophysiological measurements unveiled two new pyrethroid-sensing residues in PyR1 and four such residues in PyR2. Taken together, the new and previously published data support the following conclusions. (i) PyR1 is formed by helices IIIS4-S5, IIIS6, and IIIS6. PyR2 is formed by helices IIIS4-S5, IIIS 5, IIIS6, and IIIS6. (ii) Helix IIIS6 contains four residues that contribute to PyR1 and four residues that contribute to PyR2. (iii) Seven pairs of pyrethroid-sensing residues are located in analogous positions of domain interfaces I/I and II/III indicating rotational symmetry of the two pyrethroid receptor sites. (iv) Pyrethroids bind to both sites in similar orientations, deeply penetrating in the respective domain interfaces. Our study elaborates the dual pyrethroid-receptor sites model and provides a structural background for rational development of new pyrethroid insecticides. Supported by NIH and NSERC.

**2902-Pos Board B332**

Nav1.7 Inhibitor, PF-05089771, Inhibits Fast- and Slow-Inactivated Channels with Similar Affinities

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Voltage-gated sodium channel (Nav) inhibitors are used clinically as analgesics and local anesthetics. However, the absence of Nav channel isoform selectivity of current treatment options can result in adverse cardiac and CNS side effects, limiting their therapeutic utility. Human hereditary gain- or loss-of-pain disorders have demonstrated an essential role of Nav1.7 sodium channels in the sensation of pain, thus making this channel an attractive target for new pain therapies. We have identified a novel, human Nav1.7 selective inhibitor (PF-05089771, IC50 = 11 nM) that preferentially interacts with, and stabilizes, inactivated conformation(s) of the channel via an interaction with the voltage-sensor domain (VSD) of Domain 4. The current study demonstrates that PF-05089771 exhibits concentration-dependent slowly developing inhibition (tau = 209 sec and 33 sec, at 100 nM and 1 \( \mu \)M, respectively), and a similarly slow recovery from block upon washout (tau ~7 min). PF-05089771 exhibits minimal use-dependent inhibition until concentrations exceed 10-fold the IC50, which is consistent with the observed slow onset of block and/or a low affinity for resting or fast-inactivated channel conformations. To evaluate this further, we employed whole cell patch clamp protocols to separate channels into predominantly fast- or slow-inactivated Nav populations. Inhibition of PF-05089771 develops with similar rates using protocols that biases for either fast- or slow-inactivated states, suggesting that preference for a particular inactivated state (fast, intermediate or slow) appears less critical than the relative time that the channel is in an inactivated state during

**2899-Pos Board B329**

Loss of Calmodulin-Mediated Regulation of Na\(^+\) Channel Causes Remodeling of Electrical and Junctional Proteins; and Induces Dilated Cardiomyopathy in IQ/AA\(^+\) Mice

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Sodium channel mutations near the IQ and EFL motifs in the carboxyterminal (CT) domain have been linked to long QT (LQTS) and Brugada syndromes (BrS). IQ-calmodulin (CaM) interaction is important for regulation of cardiac Na channels. The aim of this study was to assess the role of Na\(^+\)-Ca\(^2+\)/CaM signaling via IQ motif of the Na\(^+\) channels in development and maturation of intercalated disc (ID). We studied transgenic mice with alanines knocked into IQ positions in the Na, 1.5 CT. The homozygous mice are embryonic lethal and heterozygous mice (IQ/AA\(^+\)/mice), develop cardiomyopathy (DCM). We measured the signal and distribution of Na,1.5, syntrphin, Cx43 and ryanodine in 3 and 9 month old IQ/AA\(^+\)/mice. Results were compared to those obtained from age-matched wild type mice. By immunohistochemistry we show that Na,1.5 protein in 9 month-old IQ/AA\(^+\)/mice is significantly reduced as compared to the ID. Na channel in the membrane is not altered, Cx43 which is co-localized with Na,1.5 at the ID, is significantly reduced. The expression of these proteins were not altered in 3 month-old IQ/AA\(^+\)/mice. We also assessed the implication of IQ domain on the localization of Ca\(^+\) handling protein such as ryanodine receptor and found that it was significantly altered in 9 month-old IQ/AA\(^+\)/mice. The data suggest that enhanced late Na\(_{\text{in}}\) in IQ/AA\(^+\)/mice contributes to DCM via remodeling of electrical and junctional proteins and demonstrate a dynamic interplay of Na\(^+\)-Ca\(^2+\)/CaM signaling via IQ motif of the Na\(^+\) channels in ID development and maturation. Our study highlights the importance of Ca\(^2+\)/CaM-mediated regulation of Na\(^+\) channels in DCM and arrhythmia.

**2900-Pos Board B330**

Mutation Specific Drug Response and Cardiac Risk in Long QT Type 3

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Long QT type 3 (LQT3) is caused by mutations that cause an increase in the cardiac sodium current during late phases of the cardiac action potential. For a number of LQT3 mutants this is caused by a failure to inactivate fully, and a consequent increase in late sodium current. For several mutations, shifts in voltage dependence of activation and inactivation cause increased sodium channel contribution to more depolarized voltages, an effect referred as increase in window current. The goal of this study was to perform a study in a large number of LQT3 associated mutations and compare the biophysical effect of the mutations on LQT3 patients and response to treatment. We measured the function of eight common mutations associated with LQT3 with different mechanism underlying channel dysfunction. We compared the functional effects of the channel with the clinical course of these patients and observed that for the two mutations tested with increased sustained current but without increase in current availability (window current), D1784K and D1790G, the patients had significant lower risk of cardiac events, suggesting an increase in current availability may be associated with increased risk for these patients. In addition, we measured mutation specific effects of ranolazine for these mutants. For all mutants tested, ranolazine preferentially blocked late sodium currents. Ranolazine shifts steady-state availability of the inactivation process was dependent on the specific mutant tested. Our results suggest that ranolazine may have a mutation dependent effect. Mutations dysfunction may affect drug binding and drug actions in the channel and may alter treatment effectiveness in patients.
compound exposure. The inhibition profile of PF-05099771 suggests that a
conformational change in the Domain 4 VSD couples to multiple downstream
inactivated states and immobilizing the voltage-sensor via a small molecule
interaction with this site may lock the channel into long term inactivation
from which recovery is slow.

2903-Pos Board B334
Structural Modeling of Local Anesthetic Binding to the Pore-Domain of
Human Nav1.5 in Open and Closed States using Rosetta Kevin DeMarco
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Mutations in voltage-gated sodium (Nav) channel isoforms are correlated with a
wide range of cardiovascular and neurological diseases in humans, and are therefore
are important targets for the rational design of novel drugs. The
cardiac isoform of the Nav channel, Nav1.5, presents a unique target for the
development of antarrhythmic drugs. In this work, we identify key structural
motifs for local anesthetic binding in the pore-domain of the open and closed
states of the human Nav1.5 channel. The Rosetta structural modeling method
was used to construct models of human Nav1.5 isoform based on the 3D crystal
structures of bacterial Nav channels: NaVRh (closed state) and NavMs (open state).
The resulting lowest free-energy models were selected for local anesthetic
 docking simulations. Rosetta loop modeling and global relaxation of 10,000 models yielded a convergent motif in the selectivity filter region of a
stabilizing hydrogen bond network between Tryptophan and Threonine pairs.
A membrane-facing fenestration near the S6 helix of domain IV and the S5 he-
lix of domain III was also structurally conserved, and is a proposed site of
neutral drug entry. Docking simulations of the channel blocker, lidocaine,
reveal key protein-ligand binding configurations within the pore. Our prelimi-
nary models of the human Nav1.5 channel in the open and closed states reveal
highly conserved structural motifs important for both stabilization of the pore
domain, as well as for drug entry and binding. Future work will use structural
models of drug interaction with human Nav1.5 as a dynamic testing platform
for the calculation of the kinetics of drug binding and unbinding.

2904-Pos Board B334
Understanding the State Dependence of Voltage Sensor Toxin Action on
Voltage Gated Sodium Channels Phuong T. Nguyen1,2, Ian H. Kimball1, Kenneth S. Eum1,2, Bruce E. Cohen2, Jon T. Sack1,2, Vladimir Yarov-Yarovoy1,2
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Voltage gated sodium (Nav) channels are responsible for initiation and propa-
gation of action potentials in nerve and muscle. Due to their physiological roles, Nav channels are prime targets of natural toxins from a variety of organisms such as spiders, scorpions, snakes and cone snails. ProTx-II, from the tarantula Thrixopelma pruriens, is a 30-residue peptide toxin that is a potent inhibitor of Nav1.7 channels. It binds to voltage sensor domain II (VSDII) and IV of human Nav1.7 channels. It is more than 100-fold selective for Nav1.7 versus all other
human Nav channel isoforms. Magi-5, from Macrothele gigas, is a 29-residue
peptide toxin that stabilizes an activated state of the domain II VSD of Nav
channels. Both spider toxins share a structural fold stabilized by the same disul-
fide bridge network, yet they have opposite effects on Nav function: ProTx-II stabilizes a resting state of VSDII, while Magi-5 stabilizes an activated state.
We use solid phase peptide synthesis to generate ProTx-II - Magi-5 chimeras by inserting loop regions between conserved cystines of Magi-5 into ProTx-
II. Molecular modeling, protein-protein docking and electrophysiology tech-
niques are used to identify critical residues responsible for opposite effects of
ProTx-II and Magi-5 on Nav channel function. Our findings may be useful in the
design of novel modulators of human Nav1.7 channel and may elucidate
important structural determinants of VSD toxin activity.

2905-Pos Board B335
Targeting Protein:Protein Interaction Sites for Drug Development against
Voltage-Gated Sodium Channels Syed R. Ali1, Zhiqui Liu2, Mirosław N. Nenov1, Neli I. Panova-Elektro1, Jia Zhou2, Sveta Stoilova-McPhee1, Fernanda Laezza2,1
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Fibroblast growth factor 14 (FGF14) is a functionally relevant accessory protein of
the neuronal Nav channel. Through a monomeric interaction with the intra-
cellular C-terminus of neuronal Nav channels, FGF14 modulates Na+ currents in a
Nav isoform-specific manner serving as a fine-tuning regulator of excit-
ability. In previous studies we have reconstructed the PPI interaction of
FGF14 and Nav1.6 in live cells using the split-luciferase complementation assay
(LCA) and through site-directed mutagenesis identified “hot-spots” at the FGF14
surface critical for binding to Nav1.6. Based on the FGF14 monomer structure
generated in silico, we have designed short peptide fragments that align with
pockets defined by the FGF14 β12-strand and β8-9 loop and validated their
in-cell activity as inhibitors of the FGF14:Nav1.6 complex. We then applied
patch-clamp electrophysiology and show exciting preliminary data indicating
that two peptides, Pfp1 and Epep1, exhibit either a negative allosteric modu-
lators (NAM)-like or a positive allosteric modulators (PAM)-like activity
against Nav1.6-encoded currents. For one peptide, Epep1, we have begun de-
moling its discovery efforts to generate small molecule analogs that are currently
being evaluated. These breakthrough results identify the FGF14 β8-9 and
β12 as part of potential druggable pockets against the FGF14:Nav1.6 complex
and indicate that small molecule inhibitors (SMI) and/or peptidomimetics
targeting these pockets might give rise to a new class of unconventional PPI-
based allosteric modulators of Nav channels that could restore dysfunction of
neuronal excitability and plasticity in brain disorders. These results provide
fundamental new knowledge for the design of new leads targeting the Nav chan-
nel macromolecular complex. We expect our studies to have a broad impact
in the drug design against a wide range of still untreatable brain disorders.