electron microscopy (EM) and super-resolution microscopy (gSTED, STORM) and used optical mapping and computer modeling to investigate the implications for ephaptic conduction in the heart. gSTED and STORM revealed Nav1.5 and β1 enrichment within ID regions not containing dense clusters of Cx43 and N-Cadherin. Notably, both were identified within the perinexus, a microdomain surrounding Cx43 gap junctions. Overall, 22% of Nav1.5 was located within perinexal regions while only 2% was within Cx43 clusters. EM revealed closer membrane apposition at perinexal (<10nm) vs. non-perinexal intercalated disk sites (>10nm) under control conditions. AIE increased intermembrane distance at perinexal, but not at non-perinexal sites. Functionally, this correlated with decreased transverse conduction velocity (CV-T; 15.2 ± 0.3 vs. 19.6 ± 0.1cm/s) and increased anisotropic ratio (AR; 3.0 ± 0.2 vs. 2.8 ± 0.1) relative to control, in perfused guinea pig ventricles. Next, we investigated AIE effects on Nav1.5 function in conduction. Nav1.5 blockade (0.5 μM flecainide) by itself decreased CV (18%) without changing AR. However, Nav1.5 inhibition during AIE preferentially decreased CV-T (13.0 ± 0.6cm/s), increased AR (3.3 ± 0.2) and increased spontaneous arrhythmias (7/9 vs. 4/11) compared to AIE alone. Notably, only a computer model including ephaptic coupling and the ID localization of Nav1.5 could recapitulate these results. In summary, sodium channel complexes localized to ID microdomains such as the perinexus may enable ephaptic conduction in the heart. Further, Nav1.5 functional availability and perinexal membrane spacing emerge as novel determinants of anisotropic conduction.

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

Rosy Joshi-Mukherjee[1], Hana Cho[2], Takeshi Aiba[3], Deborah DiSilvestre[1], Gordon F. Tomaselli[1].
[1]Medicine, Johns Hopkins Medical University, Baltimore, MD, USA, [2]Sungkyunkwan University School of Medicine, Suwon, Korea, Democratic People's Republic of, [3]National Cerebral & Cardiovascular Center, Osaka, Japan.

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 $Na_v1.5$ CT. The homozygous mice are embryonic lethal and heterozygous mice (IQ/$AA^{+/-}$ mice), develop cardiomyopathy (DCM). We measured the signal and distribution of $Na_v1.5$, syntrophin, 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 $Na_v1.5$ protein in 9 month-old IQ/$AA^{+/-}$ mice is significantly reduced at the ID. Syntrophin that traffics Na channels to the membrane, is not altered. Cx43 which is co-located with $Na_v1.5$ 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^{2+} 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 $I_{Na,L}$ 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

Elsa Ronzier, Yitschak Biton, Alessandra Matavel, Arthur Moss, Wojciech Zareba, Coeli Lopes.
University of Rochester, Rochester, NY, USA.

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 mutation to cardiac risk in 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 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

Yuzhe Du[1], Yoshiko Nomura[1], Ke Dong[1], **Boris S. Zhorov**[2,3].
[1]Michigan State University, East Lansing, MI, USA, [2]McMaster University, Hamilton, ON, Canada, [3]Sechenov Institute, RAS, St. Petersburg, Russian Federation.

Pyrethroid insecticides 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., 2013) located, respectively, in the II/III and I/II domain interfaces. The models differ in the number of contributing transmembrane helices, orientation of the bound pyrethroid molecules, and the depth of their penetration in respective 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 IIS4-S5 and transmembrane helices IIS5, IIS6 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 IIS4-S5, IIS5, IIS6, and IIIS6. PyR2 is formed by helices IS4-S5, IS5, IS6, and IIS6. (ii) Helix IIS6 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/II 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

Jonathan Theile, Matthew Fuller, Mark Chapman.
Pfizer - Neusentis, Durham, NC, USA.

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 μM, 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 by 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