occupancy of the intrapore receptor. The fraction of long-lived states was tightly linked with the degree of receptor occupancy. The findings provide strong support for the *m* gate trapping of LIDO.

1268-Pos Board B112

Molecular Determinants of μ-Conotoxin KIIIA Block of Voltage-Gated Sodium Channels

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mu-Conotoxin (muCTX) KIIIA is of special interest both functionally and structurally because (1) it blocks neuronal voltage-gated sodium (Na_v) channels involved in pain signalling (Zhang et al., 2007, J. Biol. Chem.) and (2) unlike previously discovered muCTXs (most >22 amino acids), KIIIA has only 16 amino acids, missing amino acids in the N-terminal section. We have performed preliminary molecular dynamics simulations of muCTX KIIIA docking to a model of the $Na_v1.4$ outer vestibule (Choudhary et al, 2007, Channels). The results are consistent with a possible binding orientation in $Na_v1.4$ with K7 facing down into the pore, interacting with the outer ring charges (E403 & E758) in domains I and II. To exam this possible orientation, single-channel bilayer recordings from rat brain (preparation includes $Na_v1.1$, 1.2, 1.3 and 1.6) and rat skeletal muscle (muscle, predominantly $Na_v1.4$) preparations demonstrated that when lysine-7 (K7) is neutralized, channels show an increase in fractional residual current (f_{res}) upon KIIIA[K7A] addition (brain, $48 \pm 3\%$ & muscle, $45 \pm 9\%$) compared to wild type KIIIA (brain, $19 \pm 3\%$, muscle $19 \pm 3\%$). The wild-type non-zero f_{res} hints that the lack of N-terminal residues or the use of a lysine residue (instead of arginine) to occlude the pore in KIIIA leads to incomplete toxin block, suggesting KIIIA has a ''looser'' interaction with the channel, with the key basic residue, K7, playing a smaller role in toxin block than in GIIIA and PIIIA. This data is supported by whole-cell experiments looking at KIIIA and KIIIA[K7A] interactions with multiple Na_v isoforms. The single-channel and whole-cell data suggest KIIIA binds to the outer vestibule with the lysine at position 7 blocking current through the pore, similar to R13 in GIIIA (skeletal muscle specific) and R14 in PIIIA (blocks both skeletal and neuronal channels).

1269-Pos Board B113

ProTxII Interacts Specifically with the Domain II Voltage Sensor of $Na_V1.4$ Modifying Gating Without Immobilization

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ProTxII, a peptide extracted from the venom of the tarantula *Thrixopelma pruriens*, binds to multiple voltage-gated sodium channel isoforms. In $Na_V1.2$ ProTxII reduces the sodium conductance and decreases total gating charge (Sokolov et al., Mol. Pharm. 73:1020). In this isoform the toxin's effect could be partially reversed with strong depolarization indicating interactions with one or more of the voltage sensors. Mutagenesis data further implicated the domain II voltage sensor in the toxin-channel interaction in $Na_V1.2$. Here we have shown that, as was seen for $Na_V1.2$, ProTxII (2-5μM) produced a decrease in maximum conductance (~60%) and a decrease in total gating charge (~20%) in the $Na_V1.4$ isoform expressed in Xenopus oocytes. Unlike $Na_V1.2$, however, these effects on $Na_V1.4$ could not be reversed with strong depolarization. We also discovered a single residue, S660, located at the S3-S4 linker/S4 boundary of DII, which when mutated, renders this channel insensitive to toxin. We used site-specific fluorescent measurements to determine the effect of ProTxII on the movement of individual voltage sensors. In the presence of ProTxII the voltage dependence of the fluorescent signal of DIIS4 was modified, but not eliminated, indicating that this voltage sensor is not completely immobilized upon toxin binding. The fluorescent signals measured from domains I and III were not significantly affected by ProTxII implicating a specific interaction with DII in producing the effect of ProTxII on $Na_V1.4$. Supported by GM30376 (FB) and NS061535-01(GBE).

1270-Pos Board B114

Tryptophan Scanning Mutagenesis to Identify the Residues Involved in Coupling between the Pore and DIII Voltage-Sensor of a Sodium Channel

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In response to membrane depolarization, voltage-gated ion channels undergo a structural rearrangement that moves the voltage sensing segments in the electric field and initiates a series of conformational transitions that ultimately opens the channel pore. The mechanism of coupling between the voltage-sensing domain and pore domain remains poorly understood. To characterize the molecular basis of this coupling, we have systematically substituted tryptophan residues in the S4-S5 region (from C-terminus of S4 to N-terminus of S5) and C-terminus of S6 of domain III in the skeletal muscle sodium channel. The effects of these perturbations on the movement of the voltage-sensor were monitored by using a site-specific fluorescent reporter on S4 of domain III using voltage-clamp fluorometry. Conformational changes in the pore were tracked by measuring the inward sodium currents. Our study identifies a number of mutants, which stabilize the voltage-sensors in the activated conformation while destabilizing the open pore conformation relative to the wild type. We suggest that the residues at these positions play an important role in coupling the voltage-sensor of domain III to the pore of the sodium channel.
Support: National Institutes of Health, American heart Association and Shaw Scientist Award.

1271-Pos Board B115

Double Mutant Perturbation Analysis Reveals High Conformational Stability Of The Domain IV S6 Segment Of The Voltage-gated Na Channel

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The S6 segment of domain IV (DIV-S6) of the voltage-gated Na channel is considered to be a key player in gating and local anesthetic drug block. Thus, some mutations in DIV-S6 substantially alter the channel's inactivation properties. In order to get a comprehensive picture of the kinetic role of DIV-S6 in fast inactivation we performed a cysteine scanning analysis of sites 1575-1591 in the DIV-S6 of the rNav1.4 channel. In addition, we produced the same cysteine replacements in the background of the mutation K1237E. K1237 is located in the P-loop of domain III and mutations at this site have dramatic effects both on permeation and gating properties. Hence, K1237E most likely causes a complex conformational change of the channel. We sought to explore whether K1237E changes the pattern of gating perturbations by the serial cysteine replacements in DIV-S6. The constructs were expressed in *Xenopus laevis* oocytes and studied by means of two electrode voltage-clamp. The half-point of availability following a 50 ms conditioning prepulse (V05) was -44 ± 1 mV and -51 ± 1 mV in wild-type and K1237E, respectively ($P < 0.001$) . Most serial amino acid replacements in DIV-S6 produced shifts in V05, both in wild-type and in K1237E background, ranging from +17 ± 1 mV to -9 ± 2 mV. A plot of the shifts in V05 by single DIV-S6 mutants relative to wild-type versus the shifts in V05 by double mutants relative to K1237E showed a significant positive correlation ($R = 0.72$, $P = 0.002$). This indicates that the general pattern of gating perturbations in DIV-S6 is not affected by K1237E, suggesting a high conformational stability of the DIV-S6 segment during the fast inactivated state. Support: FWF P21006-B11

1272-Pos Board B116

Charge Immobilization From The Open And Closed States Of Voltage-Gated Sodium Channels

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We compared the immobilization of voltage sensor gating charge during open- and closed-state fast inactivation in skeletal muscle sodium channels. To do this we compared charge movement and its immobilization using ionic and gating current recordings in the cut open oocyte configuration. Charge movement and its immobilization were steeply voltage dependent at subthreshold voltages. Kinetics of charge immobilization during open- or closed-state fast inactivation were more rapid than the concomitant decay of ionic current. The extent of charge immobilized was correlated with the completion of closed-state fast inactivation at the most negative pulse commands, and reached a maximum of 2/3 of the ON gating charge. Anthopleurin-A decreased the votlage dependence of charge immobilization compared to wild type channels. Whereas anthopleurin slowed open-state fast inactivation, the toxin accelerated currrent decrement at voltages for which only closed-state fast inactivation was possible. Anthopleurin selectively accelerated remobilzation of the gating charge in fast inactivated channels without slowing the onset or decreasing the extent of charge immobilized. This work was supported by NIH P20RR016454 to ISU.

1273-Pos Board B117

Structure-activity Relationship of Primaquine and Sodium Channel rNav 1.4

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Mutations in the putative selectivity filter region of the voltage-gated Na^+ channel, the so-called DEKA-motif not only affects selectivity and the

channel's gating properties, but also influences the affinity of antiarrhythmic drugs and controls their access to the site of union. Our lab showed that the primaquine (PQ) has an effect on cardiac native rat channels, leaving open the possibility of clinical use of the PQ as an antiarrhythmic drug. Furthermore, we studied the electrophysiological effects of PQ in Na^+ channels of $Na_v1.5$ and the $rNa_v1.4$, both with subunit β1, expressed in oocytes from *Xenopus laevis*. Those results showed that there are significant differences in the affinity of the PQ to different voltage-gated Na^+ isoforms. Recently we modeled *in-silico* interaction between the PQ and the Na^+ channel (using Autodock 3), we found that the union is likely between the drug and the lysine of DEKA-motif. Here, we tested whether charged DEKA-motif residues other than K1237 were also important determinants of the PQ interaction. Therefore, we used cysteine scanning of the DEKA-motif; D400C, E758C, K1237C, and A1529C and studied the effects of these mutations on the drug interaction. We found that compared to $rNa_v1.4$ channels, PQ on mutants E758C and K1237C had the same effect as wild type, but D400C and A1529C increased the drug potency. This suggests that mutations at position 400 and 1529 in the P-loop of domain I and IV respectively are important for the interaction between PQ and the $rNa_v1.4$.

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1274-Pos Board B118

Tetrodotoxin-sensitive Sodium Channels Contribute Significantly To The Cardiac Sodium Current In Dog Ventricles

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Introduction: The role of the late sodium current (INaL) in hereditary sodium channelopathies such as Long QT syndrome (LQTS), Epilepsy and muscoloskeletal diseases has been well characterized. In most of these cases the sodium channel defect causes an increase in the sustained component of the sodium current, INaL that significantly delays the repolarization of the target cells and tissues action potential. Interestingly, some of these neuronal and skeletal muscle diseases also display a clinical phenotype of prolonged QT interval on the electrocardiogram and cardiac rythm disturbance. These observations combined with others made since the 1970.s indirectly suggest that a tetrodotoxin-sensitive (TTX) component contributes to cardiac INaL.

Methods: We investigated the contribution of TTX-sensitive sodium channels (tNaVs) to INaL using patch clamp techniques and selective blockade of the cardiac sodium channel isoform NaV1.5 in dog ventricular myocytes. The thiosulfonate reagent (2-aminoethyl) methanethiosulfonate (MTSEA) binds to a specific cysteine in the pore region of NaV1.5 and selectively blocks this isoform. We looked at the distribution of tNaVs within the epicardial, midmyocardial and endocardial layers of the left venricle myocytes. Our results show that tNaVs contribute up to 40.18 ± 8.30 % of the late sodium current in dog cardiac myocytes. Immunoblot and mRNA data show that the molecular correlates of tNAVs: NaV1.1, NaV1.2 and NaV1.4 account for a significant portion of this contribution.

Conclusions: We conclude that tNAVS are present in the cardiac ventricles of higher order mammals. In man, such contribution to INaL could explain the incidence of cardiac arrhythmias and QT prolongation observed in neuronal and musculoskeletal diseases and some of the cardiac secondary effects of neuroleptic drugs.

1275-Pos Board B119

Calcium Signalling By Sodium Channels In Developing Rabbit Cardiomyocytes

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Recent studies have demonstrated that in the neonatal cardiomyocytes, Ca^{2+} influx through reverse-mode NCX activity is sufficient to induce calcium induced calcium release. This study is undertaken to study the molecular components of excitation-contraction coupling in neonatal cardiomyocytes. The expression of voltage gated sodium channels was determined using Western blot analysis at different developmental time points. In this study, we investigate the regulation of neuronal ($Na_v1.1$, $Na_v1.3$, $Na_v1.6$), skeletal (($Na_v1.4$) and the cardiac $Na_v1.5$ isoforms and their respective intermolecular interactions with NCX in developing hearts. Immunoblot analysis of heart samples isolated from rabbits at 3, 10, 20 and 56 days after birth revealed a robust expression of skeletal muscle ($Na_v1.4$) in the neonates and decreases significantly in 56 day old rabbit. The neuronal isotypes Na_v 1.1 and $Na_v1.3$ were found to have low levels of expression through development. Cardiac isoform (Na_v 1.5) expression was similar to Na_v 1.4 in the neonatal heart homogenates but the protein levels decreased in the 56 day heart homogenate. In isolated cardiomyocytes, skeletal isoform protein expression was significantly more prominent in neonates (3 days) compared to the adult (56 day). Our preliminary results suggest that in the neonate heart $Na_v1.4$ may dictate the role of NCX in regulating Ca^{2+} influx during contraction.

1276-Pos Board B120

A Common SCN5A Polymorphism Restores the Biophysical Defects of LQT3 Mutations

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Mutations in *SCN5A* cause inherited susceptibility to ventricular arrhythmias such as Long QT syndrome (LQTS). We recently found a family which exhibited an unusual LQT3 syndrome phenotype. Although, the *SCN5A* mutation (P2006A) was found, there are no clinical sign of LQTS at rest. Additionally, the patients were found to be homozygous for a common sodium channel polymorphism H558R. In HEK293 cells, P2006A displayed a typical pathological signature of the LQT3 phenotype. Interestingly, when the mutation was co-expressed with the H558R polymorphism, the sodium currents behaved like wild-type (WT). Given that the polymorphism entirely restored the biophysical defects caused by the P2006A mutation, we considered whether residual kinetic changes due to the interaction between the *SCN5A*-H558R and *SCN5A*-H558R-P2006A could explain the mild phenotype seen in the patients who are homozygous for H558R and heterozygous for P2006A. Co-expression of *SCN5A*-H558R with *SCN5A*-P2006A or *SCN5A*-H558R-P2006A in HEK293 cells were characterized using the patch-clamp technique. Here we show that *SCN5A*-H558R can mitigate the in vitro gating defects caused by *SCN5A*-P2006A explaining the absence of typical LQT3 phenotype in the family members carrying the H558R polymorphism in addition to the P2006A LQT3 mutation. Moreover, we investigated whether H558R can also modulate fast inactivation in other LQT3 mutations located in the C-terminus of *SCN5A*. The V1950L mutation causes depolarizing shift in steady-state inactivation and produces a long QT phenotype. Once again, the double mutation *SCN5A*-H558R-V1950L restored the gating defects to the WT level, suggesting that H558R might play an important role in stabilization of channel inactivation. These results not only point to a modulatory effect of the H558R polymorphism on the fast inactivation gating characteristics of these LQT3 mutations, but may provide a plausible mechanism for the variable penetrance seen in several LQT3 families.

1277-Pos Board B121

SCN5A Missense Mutation from a Patient with Complex Cardiac Rhythm and Conduction Disorder Requires the Common Polymorphism H558R on the Same Allele for Arrhythmogenic Biophysical Phenotype

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Background: Mutations in *SCN5A* that decrease peak I_{Na} cause several arrhythmogenic syndromes such as type 1 Brugada syndrome (BrS1), cardiac conduction disease (CCD), and congenital sick sinus syndrome (SSS). Here, we report a novel missense mutation (V240M) in *SCN5A,* in the presence of the common polymorphism H558R that perturbs cardiac rhythm and conduction. Methods and results: A 10-year-old boy had atrial fibrillation and sinus pauses up to 6 seconds during 24h Holter monitoring. Nine months after pacemaker implantation, monomorphic ventricular tachycardia (VT) was recorded during exercise. He was diagnosed clinically with SSS, CCD and VT. Comprehensive open reading frame/splice site mutational analysis of *SCN5A* was performed using DHPLC and DNA sequencing. A missense mutation (V240M), localized between the DI-S4 and DI-S5 region of the sodium channel, and the common polymorphism H558R were found on the same allele. The double mutation was engineered by site direct mutagenesis and expressed in HEK cells for voltage clamp study. After 24h of transfection, the current densities of SCN5A-V240M were reduced compared with WT channels (-175 ± 27 pA/pF for V240M and -417 ± 100 pA/pF for WT), after 48h of incubation, the current densities of SCN5A-V240M were comparable to WT levels. However, SCN5A-V240M/H558R had current densities dramatically reduced (-34 ± 17 pA/pF). In addition, gating kinetic analysis showed a 10 mV negative shift of inactivation and slower time constants of recovery, all of which would tend to reduce peak I_{Na}. Conclusion: The profound biophysical phenotype with loss of function could account for the severity of the clinical phenotype. The requirement for H558R represents another example, and a dramatic one, of phenotype modification by this common polymorphism.