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 $rNa_v1.4$.

Acknowledgments: BUAP-VIEP SASE-SAL-08-G, CONACYT #48294-M and CONACYT #175906 to EMM.

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.

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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.

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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.

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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.