acidic residues in DPP6-S eliminated the increase in γ. Therefore, DPP6-S, as a membrane protein extrinsic to the pore domain, is necessary and sufficient to explain a fundamental difference between native and recombinant Kv4 channels. These observations may help to understand the molecular basis of neurological disorders correlated with recently identified human mutations in the *dpp6* gene.This work was supported by grants from the National Institutes of Health (R01 NS032337-13 to MC; and NS045217 and NS30989 to BR).

919-Pos Board B798

Cardiac Kv4.3 and KCNE2 Are Differentially Regulated by E2 and Have Different Sensitivities to Local Heart E2 Concentrations

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Recently we reported that the KCNE2 gene is an estrogen-responsive gene and its transcripts are upregulated 6 fold by estrogen (E2) in ovariectomized (ovx) mice[1]. We have also shown that cardiac Kv4.3 transcripts were downregulated ~2 fold by E2[2]. As the effect of E2 treatment was more powerful on KCNE2 upregulation than Kv4.3 downregulation, we hypothesized that cardiac Kv4.3 and KCNE2 have different sensitivities to heart E2 concentrations [E2]. We measured heart [E2] together with KCNE2 and Kv4.3 transcript levels in 4 estrogenic conditions: i) E2-depleted (anastrozole treated mice, [E2]=4.2 ± 0.4 pg/ml n=4; ii) low E2 (ovx sham, [E2]=16 ± 1.4 pg/ml n=3 and diestrus [E2]=20.2 ± 1.5 n=4), iii) intermediate E2 (male [E2]=35 ± 3 pg/ml, n=6) and iv) high E2 (ovx mice treated with E2, [E2]=62.7 ± 2.9 pg/ml, n=3). Kv4.3 transcript levels were not affected by heart [E2] lower than 35 pg/ml whereas KCNE2 transcript levels were very sensitive to this range of heart [E2], reaching a ~10 fold increase from low to intermediate heart [E2], saturating at 35 pg/ml. The fact that Kv4.3 levels were unaffected by anastrozole treatment, whereas KCNE2 levels were dramatically reduced by ~8 fold by anastrozole, further supports the finding that KCNE2 upregulation can take place at very low E2 levels. The downregulation of Kv4.3 transcripts were only evident at high estrogenic conditions, whereas KCNE2 remains at its maximum. As Kv4.3 is one of the molecular correlate of $I_{to,f}$ and it has also been shown that KCNE2 can potentiate Kv4.3 currents in the expression system, we speculate that the relative expression of KCNE2 and Kv4.3 as defined by heart [E2] will determine $I_{to,f}$ amplitude.

1. Kundu et al., *Mol Cell Endocrinol* 2008;292:50-62.
2. Eghbali et al., Circ Res 2005;96:1208-16.

920-Pos Board B799

Vernakalant Blocks Kv4.3 Channels in The Open State Without Significant Modulation by KChIP2 Subunits

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Vernakalant, a relatively atrial selective mixed ion channel blocker, rapidly converts atrial fibrillation to normal sinus rhythm in humans. Previous studies demonstrated that vernakalant blocks Kv4.3 but the state dependence of blockade and influence of KChIP2 were not determined. Kv4.3 ± KChIP2 was transfected in HEK cells and currents were recorded by whole-cell voltage clamp.

Measured activation and inactivation kinetics and voltage dependence was consistent with current models of closed, open, open-inactivated, and closed-inactivated states. Vernakalant, with little effect on peak current (τ_{act} = 0.39 ± 0.02 ms), induced a very rapid initial decay (τ_{ass} = 3.90 ± 0.21 ms) followed by the well-described fast (τ_{fast} = 39.9 ± 4.1 ms) and slow (τ_{slow}= 193 ± 22 ms) components of steady state inactivation. This indicates that vernakalant rapidly associates with the open state causing block (IC_{50} = 23.0 ± 5.1 μM). Tail currents following short periods of depolarization (+10 mv, 10 ms) insufficient to induce inactivation, initially decayed more rapidly in the presence of vernakalant. However the slow time constant (τ_{diss} = 33.1 ± 5.9 ms, n=2) was much longer than deactivation (τ_{deact} = 14.8 ± 1.8 ms, n=2) leading to crossover of tail currents. Thus, vernakalant rapidly associated with the open state to produce a drug blocked state and less rapidly dissociated back to the open state, which then deactivated to the closed state. Vernakalant did not affect recovery from inactivation. Co-expression with KChIP2 did not affect vernakalant's potency (IC_{50} = 22.3 ± 4.7 μM). This is consistent with previous studies showing that KChIP2 modulates inactivation kinetics with little effect on activation kinetics.

In conclusion, vernakalant rapidly blocks Kv4.3 in the open state and KChIP2 does not modulate the Kv4.3 block by vernakalant.

921-Pos Board B800

Characterization of the External Sodium Inhibition of hERG Potassium Channels

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In the absence of external K^+, external Na^+ (Na^+_o) inhibited the *human ether-a-go-go-related gene* (hERG)-encoded channel K^+ current (I_{hERG-K}) with an IC_{50} of 4.4 ± 0.7 mM, and Na^+ current ($I_{hERG-Na}$) with an IC_{50} of 3.5 ± 0.4 mM ($P >$ 0.05). Using the whole cell patch clamp and site-directed mutagenesis methods on recombinant hERG channels, we found that Na^+_o-induced inhibition of hERG current was intrinsically independent of either activation or inactivation of the channel. In the absence of K^+, Na^+_o inhibited $I_{hERG-Na}$ in a time-dependent manner with a time constant of 15.0 ± 0.4 s. The recovery of $I_{hERG-Na}$ from inhibition after washout of Na^+_o was also time dependent, with a time constant of 27.1 ± 1.0 s. K^+ competes with Na^+ for binding to interfere with Na^+_o mediated inhibition of hERG channels. When I_{hERG-K} was maximally inhibited by 135 mM Na^+_o, outflowing K^+ during channel opening dynamically relived Na^+_o-induced inhibition. As well, addition of K^+ to the 135 mM Na^+-containing bath solutions relieved the Na^+_o-mediated inhibition of I_{hERG-K} with an EC_{50} of 1.2 mM. Point mutations in the hERG pore region were identified to completely eliminate hERG Na^+ sensitivity. We propose that K^+_o and Na^+_o compete for a binding site(s) close to the permeation pathway of the channel to determine the fate of hERG function. While K^+-bound hERG channels gate normally, Na^+-bounding fosters the hERG channel entering into a nonfunctional conformation.

922-Pos Board B801

Papaverine, A Vasodilator, Blocks The Pore Of The Herg Channel At Submicromolar Concentration

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Papaverine, a vasodilator used as a therapeutic agent for a range of diseases, has been reported to increase the risk of occasional serious ventricular arrhythmias. To examine the mechanism for this effect, we herein tested the effects of papaverine on human *ether-a-go-go* (HERG) K^+ channels expressed in HEK293 cells and *Xenopus* oocytes. Our results revealed that papaverine dose-dependently decreased the tail currents of HERG channel expressed in HEK293 cells with the IC_{50} and the Hill coefficient of 0.58 μM and 0.58, respectively, at +20 mV and 36 °C. The IC_{50} for the papaverine-induced blockade of HERG current in *Xenopus* oocytes was found to decrease progressively relative to depolarization (38.8, 30.0, and 24.8 μM at -10, +20, and +40 mV, respectively). The papaverine-induced blockade of HERG current was time-dependent; the fractional current was 0.92 ± 0.03 of the control at the beginning of the pulse, but declined to 0.18 ± 0.06 after 6 seconds at a test potential of 0 mV. These results collectively indicate that papaverine blocks HERG channel in a concentration-, voltage-, and time-dependent manner. Two S6 domain mutations, Y652A and F656A, partially attenuated (Y652A) or abolished (F656A) the hERG current blockade, suggesting that papaverine blocks HERG channel at the pore of the channel. This was consistent with the computational simulation that showed papaverine interacts with Tyr652 and Phe656. Therefore, ventricular arrhythmias induced by papaverine could be resulted from the blockage of the HERG channel at the cardiac myocytes.

923-Pos Board B802

Block Of The HERG Mutant D540K By Terfenadine Shows The Opposite Dependency On Extracellular Potassium Compared To Block Of WT HERG By Terfenadine

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Block of the cardiac potassium channel HERG by a number of drugs has been shown by different investigators to depend on the extracellular potassium concentration. This dependency on extracellular potassium can be explained by at least two mechanisms: destabilization of the drug by the permeant ion or differential binding to the inactivated state. We previously reported that block of HERG by terfenadine shows the opposite dependency on extracellular potassium compared to quinidine. Thus HERG block by quinidine is greater in 0 mM K compared to 20 mM K whereas block by terfenadine is greater in 20 mM K compared to 0 mM K. In order to determine the mechanism underlying this difference in potassium dependency we measured block by terfenadine of the HERG mutant D540K which opens with both depolarization and hyperpolarization. Block of D540K by terfenadine showed the opposite dependency on extracellular potassium compared to block of WT HERG by terfenadine. Thus block of D540K by terfenadine is greater in 0 mM K compared to 20 mM K, similar to the extracellular potassium dependency of block of WT HERG by quinidine. Recent experiments indicate that terfenadine is trapped inside the channel after the channel closes, whereas quinidine is not[1]. In addition we have reported that block of HERG by quinidine shows a better correlation with the permeant ion than with inactivation. Together these results suggest that the permeant ion is not able to destabilize a trapped drug but is able to destabilize a drug that is not trapped and indicate a possible role for the activation gate in determining the extracellular potassium dependency of block of HERG.

[1] Stork et al. (2007) *BJP* **151**:1368-1376.