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 NS032373-13 to MC; and NS045217 and NS30989 to BR).

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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. We have also shown that cardiac Kv4.3 transcripts were downregulated ~2 fold by E2. As the effect of E2 treatment was more powerful on KCNE2 up-regulation than Kv4.3 downregulation, we hypothesized that cardiac Kv4.3 and KCNE2 have different sensitivities to heart E2 concentrations [E2]. We measured [E2] [E2] together with KCNE2 and Kv4.3 transcript levels in 4 estrogenic environments: i) E2-depleted (anastrozole treated mice), [E2]=4.2 ± 0.4 pg/ml n=4; ii) low E2 (ovx sham, [E2]=20.2 ± 1.5 pg/ml, 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 Ih,R and it has also been shown that KCNE2 can potentiate Kv4.3 from low to intermediate heart [E2], saturating at 35 pg/ml. The fact that Kv4.3

4.4 ± 0.7 M, and Na+ current (iHERG-Na) with an IC50 of 3.5 ± 0.4 nM (P > 0.05). Using the whole cell patch clamp and site-directed mutagenesis methods on recombinant hERG channels, we found that Na+-induced inhibition of hERG current was intrinsically independent of either activation or inactivation of the channel. In the absence of K+ but Na+ inhibited iHERG-Na, the IC50 for the papaverine-induced blockade of HERG channel 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 IC50 and the Hill coefficient of 0.58 μM and 0.58, respectively, at +20 mV and 36 °C. The IC50 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.

913-Pos Board B801
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 different 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 of 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. In addition we have reported that block of HERG by terfenadine 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.