conceived LQT1 at rest (QTc, 452 ± 5 ms) but shows marked paradoxical QTc prolongation (53.2 ± 16 ms) during the recovery phase of treadmill stress testing. This abnormal recovery response in the absence of drug effects suggests that patients taking β-adrenergic blockers (487 ± 11 ms). Voltage-clamping experiments using HEK293 cells expressing WT or I235N with the Kv7.1 β-subunit KCNE1 showed that I235N decreased KCNQ1 current (IKCNQ1) by 93% and caused a large positive shift in the midpoint potential for activation (V1/2). However, cells co-expressing WT and I235N (to mimic the patients’ genotypes) showed only a small decrease in IKCNQ1 (± 30%) and shift in V1/2. Since excessive QTc prolongation in I235N patients appears secondary to β-adrenergic stimulation, we tested whether I235N prevented PKA activation of IKCNQ1. In cells expressing WT, PKA stimulation with forskolin and IBMX increased IKCNQ1 by 64%, but it did not increase IKCNQ1 in cells co-expressing WT and I235N. Computational simulations using a ventricular action potential (AP) model showed that reducing the IKs component by 30% increased the AP duration at 90% repolarization (APD90) by only 1.6%. However, incorporating β-adrenergic signaling showed that reducing the IKs component by 30% and preventing its activation by PKA increased the APD90 by 7.1%. We conclude I235N modestly affects basal QTc, IKs, and APD90, but it prevents PKA activation of IKs to cause a dangerous prolongation in the QTc/APD90 during β-adrenergic stimulation.

1376-Pos Board B268
Malignant Long QT Syndrome KCNQ1 Mutations in the Pore Disrupt the Molecular Basis for Rapid K+ Permeation
Don E. Burgess1, Daniel C. Bartos1, Allison R. Reloj1, Kenneth S. Campbell1, Jonathan N. Johnson2, David J. Tester2, Sara I. Liin1, Nicole Schmitt2, Bo H. Bentzen2, Fredrik Elinder1.

1University of Kentucky, Lexington, KY, USA, 2Mayo Clinic, Rochester, MN, USA, 3UMPC Université, Paris, France, 4University of Rochester Medical Center, Rochester, NY, USA, 5Shiga University of Medical Science, Ohtsu, Japan.

Type I long QT syndrome (LQT1) syndrome is caused by loss-of-function mutations in the KCNQ1-encoded K+ channel (Kv7.1) that underlies the slowly activating delayed rectifier K+ current (IKs) in the heart. Intragenic risk stratification suggests LQT1 mutations that disrupt conserved amino acid residues in the pore are an independent risk factor for LQT1-related cardiac events. The purpose of this study is to determine possible molecular mechanisms that underlie the loss-of-function for these higher risk mutations. Extensive genotype-phenotype analyses of LQT1 patients showed that the pore mutations T322M-, T322A-, and G325R-Kv7.1 cause a loss-of-function by disrupting delayed rectifier K+ (Kv7.1) channels in CHO cells expressing KCNQ2 potassium channels. A linear model of KV gating showed that reducing the IKs component by 30% and preventing its activation by PKA increased the APD90 by 7.1%. We conclude I235N modestly affects basal QTc, IKs, and APD90, but it prevents PKA activation of IKs to cause a dangerous prolongation in the QTc/APD90 during β-adrenergic stimulation.

1378-Pos Board B270
Regulation of the Kv7.2/3 Channels by the Neuronal Serum- and Glucocorticoids-Regulated Kinase 1.1
Pablo Miranda1, Alba Cadaveira-Mosquera2, Rafaela Gonzalez-Montelongo1, Alvaro Villarreal3, Jose Antonio Lamas2, Diego Alvarez de la Rosa1, Teresa Giralle1.

1Unidad de Investigacion HUNSC, Tenerife, Spain, 2Universidad de Vigo, Vigo, Spain, 3Unidad de Biofisica/UPV/CSC, Leioa, Spain.

Voltage gated K+ channels are key regulators of neuronal excitability. The M-Current, formed by tetramerization of Kv7.2 and Kv7.3 subunits, is a voltage gated K+ current present in neurons. The inhibition of this current leads to depolarization of the membrane potential. It has been described previously that the neuronal serum and glucocorticoids-regulated kinase 1 (SGK1) induces an increase in the amount of Kv7.2/3 channels in the membrane (Schuetz et al. 2008). We now show that the neuronal isoform of this kinase (SGK1.1) induces an up-regulation of the Kv7.2/3 current in Xenopus laevis oocytes, while the kinase-inactive mutant K220A does not produce an effect. SGK1.1 interacts with PIP2 and is normally localized to the plasma membrane (Artega et al. 2002). An SGK1.1 mutant disrupting PIP2 binding (K21N/K22N/R23G; Wesch et al. 2010) had no effect in the amplitude of the Kv7.2/3 current. SGK1.1 did not modify the voltage dependence and open or close kinetics of the Kv7.2/3 channels, suggesting that the kinase alters channel abundance in the membrane. We also tested M-current amplitude in neurons of the superior cervical ganglion (SCG) isolated from transgenic mice expressing a constitutively active form of SGK1.1 (SS15D). Transgenic SCG neurons showed an increase in M-current amplitude, consistent with a trend towards a more negative resting potential and less excitability when compared with wild-type SCG neurons. Our conclusions are:

- SGK1.1 is a novel regulator of M-channels.
- SGK1.1 could be an integrator of different signal transduction pathways controlling M-channels and therefore neuronal excitability.

1379-Pos Board B271
Mechanistic Determinants of M-Resonance
Peng Jiang1, Xiaodong Liu.

Tsinghua University, Beijing, China.

Electrical resonance of excitable membranes associated with subthreshold M-current (M-resonance) has been proposed to underlie theta frequency of EEG, cognitive functions, certain forms of epilepsy, and sensory signal processing. However, the biophysical mechanisms of M-resonance remain elusive. Using whole-cell patch-clamp technique, we reproduced M-resonance phenomena in CHO cells expressing KCNQ2 potassium channels. A linear model of Kv7 channels was proposed to analytically decompose M-resonance evidenced in primary cells as well as in our heterologous expression system. Guided by our theoretical modeling, mutagenesis analyses indicated that M-resonance is tightly correlated with the unusually slow activation/deactivation of M-channels, in contrast to other Kv channels with much faster kinetics, which exhibit rather weak or no resonance. Perturbations of the key residues on the channel sustaining the unique M-kinetics also corrupted M-resonance. Taken together, the biophysical origin of M-resonance was revealed by our study, providing innovative insights into the design principle of M-channels for fulfilling its physiological functions.

1380-Pos Board B272
M-Type K+ Channel as Plasma Membrane Nitric Oxide and Reactive Oxygen Species Sensor
Lezanne Ooi, Louisa Pettinger, Nikita Gamper.

University of Leeds, Leeds, United Kingdom.

M-type K+ channels control excitability of many neuron types. M channel activity is potentially enhanced by oxidative modification of triple cysteine pocket within the cytosolic linker between transmembrane domains 2 and 3. Thus, in...