concealed LQT1 at rest (QTc, 452 ± 5ms) but shows marked paradoxical QTc prolongation (534 ± 16ms) during the recovery phase of treadmill stress testing. This abnormal recovery response in the biophysical origin of M-channels was blunted in patients taking flunarizine blockers (487 ± 11ms). 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**

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Type 1 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-, or G325R-Kv7.1 confer a higher risk for LQT1-related cardiac events. Heterologous expression of these mutations with KCNE1 suggested that they generated non-functional channels and caused dominant negative suppression of WT-Kv7.1. We performed molecular dynamic simulations (MDS) for the analogous mutants in KcsA (T85M-, T85A-, and G88R-KcsA). MDS of WT-, T85M-, T85A, and G88R-KcsA channels showed the selectivity filters form contiguous surfaces such that the T85 residue and some other residues (S1-S4) could bind to K⁺ ions and water molecules in an alternating manner. For the most part, the oxygen atoms that line the WT-KcsA selectivity filter faced the central pore axis; however, the T85M-, T85A-, or G88R-KcsA simulations showed they stabilized a configuration where the carbonyl oxygen atom between S2 and S3 was "flipped" away from the lumen of the selectivity filter. T85M- and T85A-KcsA stabilized the flipped configuration for two adjacent z-subunits. The stabilization of the flipped configuration upset the balance between the strong attractive and K⁺ repulsive forces required for rapid K⁺ permeation. We conclude that the T322M-, T322A-, and G325R-Kv7.1 cause a loss-of-function by disrupting the architectural and physical properties of the selectivity filter.

**1377-Pos Board B269**

**Polyunsaturated Fatty acids as Kv7 Channel Modulators**

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Lipids in different forms can directly affect the electric activity of excitable tissues, in some cases via direct interactions with ion channels. For instance, is the activity of the Shaker K⁺ channel dependent on the abundance of the phospholipid POP2 in the inner leaflet of the plasma membrane. In this work we instead studied the effect of free extracellular polyunsaturated fatty acids (PUFAs) on Kv7 channels expressed in Xenopus oocytes. We show that ω-3 and ω-6 PUFAs affected the voltage dependence of Kv7.1 and Kv7.2/3 by shifting the conductance versus voltage (G(V)) curves in negative direction along the voltage axis. The effect was pH dependent. In contrast, uncharged methyl esters of the PUFAs did not affect the voltage dependences. Fatty acid requirements and PUFAs-induced effects were similar to those previously reported for the Shaker K⁺ channel suggesting a similar modulatory mechanism of action, i.e. our previously proposed lipoelectric mechanism in which PUFAs electrostatically affect channel opening. The putative PUFA interaction site is close to the positioning of auxiliary KCNE subunits. We therefore also investigated the impact of KCNE subunits and found that PUFA potency was influenced by KCNE association. Kv7.1 and Kv7.2/3 channels are important for cardiac and neuronal excitability, respectively, and mutated channels resulting in loss-of-function cause heart rhythm disturbances and epilepsy. The PUFA-induced opening of Kv7 channels found in this study may therefore help explain the antiepileptic properties of PUFAs and the fat-rich ketogenic diet. Lipoelectric modification of the channels voltage dependence could be a future new approach for pharmacological treatment.

**1378-Pos Board B270**

**Regulation of the Kv7.2/3 Channels by the Neuronal Serum-and Glucocorticoids-Regulated Kinase 1.1**

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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 PI2 and is normally localized to the plasma membrane (Artzga et al. 2006). An SGK1.1 mutant disrupting PI2 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**

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Electrical resonance of excitable membranes associated with subthreshold M-current (M-resonance) has been proposed to underlie theta frequency of certain neuronal activity. The biophysical origin of M-resonance was revealed by our study, providing in-depth understanding of the mechanisms that underlie the resonance. Perturbations of the key residues on the channel pore alter the resonance, with more charges increasing the amplitude of the resonance. Perturbations of the residues on the cytosolic linker between transmembrane domains 2 and 3 disrupt the resonance, suggesting that the neuronal isoform of this kinase (SGK1.1) induces an increase in resonance amplitude. Our study provides a comprehensive understanding of the mechanisms that underlie the resonance and suggests novel targets for modulating neuronal activity.
sensory neurons from dorsal root (DRG) and trigeminal (TG) ganglia M channel activity is increased by the mitochondrial release of reactive oxygen species (ROS) induced by neuropeptide substance P. Humanized M-channel subunits express a new signalling role for nitric oxide (NO) in TG sensory neurons. We show that in rat TG neurons, the NO donor, S-Nitroso-N-acetyl-DL-penicillamine (SNAP), inhibited M-current by 53 ± 12% (n = 12). This inhibitory effect was blocked by scavenging of NO and inhibition of NO synthases increased M-current, suggesting that tonic NO levels inhibit M-current in TG neurons. NO increased release of calcitonin gene-related peptide (CGRP) from TG neurons (130 ± 10% of control, p < 0.05), consistent with an increase in neuronal excitability. Importantly, incubation with the M-channel opener retigabine completely abolished increases in CGRP release. We further investigated the mechanism of the effects of NO on M channels and identified a site of action of NO to be the same triplet of cysteines, which is also a site of oxidative modification of M channels by ROS. We now show that the same triplet of cysteines can be S-nitrosylated in the presence of SNAP. We show that NO and oxidative modifications have opposing effects on M-current, suggesting that a tightly controlled local redox and NO environment will exert control over M-channel activity and thus neuronal excitability. Together our data have identified a dynamic redox sensor within neuronal M-channels which mediates reciprocal regulation of channel activity by S-nitrosylation and oxidative modification. This sensor may play an important role in controlling neuronal excitability by redox and NO-related mechanisms.

1381-Pos Board B273
Binding of ATP is Required for Opening of I\textsubscript{Ks} Channels
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The human heart demands a constant energy supply to fulfill its function, hence the decrease in myocardial ATP production plays a key role in the pathogenesis and progression of ischemic heart diseases. Ischemia also alters electrophysiology of the heart, exemplified by an association between an adverse prognosis and QT interval prolongation in acute myocardial ischemia. KCNQ1 and KCNE1 form the I\textsubscript{Ks} potassium channel important in terminating cardiac action potentials. Congenital mutations that compromise I\textsubscript{Ks} function prolong the duration of the ventricular action potential, causing Long QT (LQT) syndrome, which is associated with a high risk of sudden death. Here we report that the I\textsubscript{Ks} channel activity increases with ATP concentration ([ATP]) and the EC\textsubscript{50} is close to the physiological [ATP] in cardiac myocytes, which indicates that [ATP] changes such as in ischemia affect I\textsubscript{Ks} channel function. Consistent with this observation, an LQT-associated mutation in KCNQ1 alters I\textsubscript{Ks} function by reducing ATP sensitivity. The effect of this mutation is eliminated by increasing [ATP]. We find that GTP and a non-hydrolyzable ATP analog AMP-PNP can substitute for ATP in activating the channel, and an ATP analog can be photo-cross-linked to KCNQ1 proteins expressed in the membrane of Xenopus oocytes, indicating that the nucleotide directly binds to KCNQ1 to modify channel function. Compared to ATP, ADP and AMP are less effective in activating the I\textsubscript{Ks} channel, suggesting that phosphate groups are important in nucleotide binding. Correspondingly, a mutational scan of all cytosolic basic residues shows that the ATP binding site may reside in the cytosolic C-terminus. These results demonstrate that I\textsubscript{Ks} is a bona fide ATP activated potassium channel, suggesting that phosphate groups are important in sensing ATP, thus connecting the cellular energy state to membrane excitability.

1382-Pos Board B274
Global Ischemia Upregulates KCNQ1 Potassium Channel Activity in Neurons
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Transient forebrain or global ischemia induces delayed cell death of hippocampal CA1 pyramidal neurons and impaired cognition. Potassium (K\textsuperscript{+}) channels are implicated as key players in ischemia-induced death. The KCNQ1 K\textsuperscript{+} channel assembles with the auxiliary subunit KCNE1 to produce the slow component of I\textsubscript{Ks} in the heart. Emerging evidence indicates that KCNQ1 mRNA and protein are expressed not only in heart, but also brain, although its function in neurons is, as yet, unclear. The transcription factor REST (repressor element-1 silencing transcription factor)/NRSF (neuron-specific silencing factor) is upregulated in response to ischemia, consistent with a possible contribution to delayed neuronal death of the CA1.

1383-Pos Board B275
Protein Kinase C-Dependent Modulation of Heterologously Expressed Homomeric Kv7.4, Kv7.5 and Heteromeric Kv7.4/7.5; Differential Sensitivities to Low Concentrations of Vasopressin and PMA in A7R5 Vascular Smooth Muscle Cells
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Kv7.4 and Kv7.5 voltage-activated potassium channels are proposed to contribute to the maintenance of resting membrane voltage in smooth muscle cells. We had previously provided evidence that Kv7.4 and Kv7.5 form predominantly heteromeric channels when natively or exogenously expressed in vascular smooth muscle cells. Endogenous Kv7 currents in smooth muscle cells are suppressed upon activation of G\textsubscript{1a} coupled receptors. It remained to be elucidated if both Kv7.4 and Kv7.5 respond similarly to low concentrations of vasopressin (AVP), a known agonist of the V1a Gq-coupled receptor. Using patch-clamp techniques, we measured currents through human Kv7.4 and Kv7.5 channels expressed individually or together in A7r5 rat aortic smooth muscle cells and compared their sensitivity to AVP (100 pM and 500 pM) and to the protein kinase C (PKC) activator phorbol 12-myristate 13-acetate (PMA, 1nM). AVP (100 pM) and PMA suppressed currents through Kv7.4 and Kv7.5 channel. Currents were reproducible with different voltage dependencies and potencies in the rank order: Kv7.5 > Kv7.4/7.5 > Kv7.4. Both AVP and PMA increased the steepness of Kv7.4 voltage-dependent activation and dramatically decreased Gmax. In contrast, AVP and PMA induced a rightward shift of the Kv7.4 activation curve with only a slight reduction in maximal conductance (Gmax). The modulation of Kv7.4/7.5 activation by AVP and PMA had intermediate biophysical characteristics that were distinct from the modulation of either of the homomeric configurations. These findings reinforce the significance of PKC-dependent regulation of the Kv7 channels and suggest a differential regulation of Kv7.4 and Kv7.5 channel subunits by PKC-dependent phosphorylation.

1384-Pos Board B276
Kcnq Channels in Airway Smooth Muscle
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The role of KCNQ (Kv7) channels is well established in neurons, where they play dominant roles in control of resting membrane potentials and cell excitability. Recent studies have revealed expression of KCNQ channels in different types of smooth muscle. Here we focused on airway smooth muscle (ASM), whose membrane potential is primarily, determined by BK-type K\textsuperscript{+} channels. RT-PCR and immunostaining suggest that KCNQ 1, 4 and 5 are the predominant subtypes in rodent airway. To investigate the contribution of KCNQ channels in cholinergic-induced ASM contraction, we patch-clamped freshly isolated ASM cells and isolated KCNQ current as the non-inactivating component at the end of 2s depolarizations, with BK channels blocked by 1mM paxilline. The KCNQ current was enhanced by the KCNQ channel opener, flupirtine (10mM), and abolished by XE991 (10mM). Although XE991 depolarized the resting membrane potential, it did not affect that of ASM cells pre-treated with carbachol. Similarly, XE991 did not effect carbachol-evoked contractions, whereas flupirtine induced a significant relaxation. The flupirtine effect is likely via opposing voltage-dependent Ca\textsuperscript{2+} influx, since no effect of flupirtine occurred in a low K\textsuperscript{+} (1mM) bathing solution. Pre-treatment with the muscarinic M\textsubscript{1} receptor antagonist, fumarate (5nM), to prevent G\textsubscript{1a} mediated PP\textsubscript{3} hydrolysis did not reveal an effect of XE991 on contractions, suggesting the mechanism of KCNQ inhibition in ASM is not due to PP\textsubscript{3} depletion. Interestingly, an XE991-dependent increase in conductivity was revealed in mice with the B1 BK channel subunit knocked out, an effect enhanced by the M\textsubscript{1} receptor antagonist. Thus, KCNQ channels may normally play a secondary role to BV1 in control of cholinergic evoked contractions in ASM; however, given that alteration in BK B1-subunit expression is known to occur in asthma, KCNQ channels in ASM cells may be potential targets for therapeutic intervention in respiratory disease.