KCNQ2 but not KNCQ3, is expressed on neuronal axons, where it might regulate action potential propagation or neurotransmitter release.
Previously, we showed that Syntaxin 1A physically interacts with homomeric KCNQ2 in brain synaptosomes and in *Xenopus* oocytes. In oocytes, this interaction results in a reduction of the current's amplitude and reduction of the channel's activation rate. In vitro pull down revealed that Syntaxin 1A specifically binds the Helix A domain located in the C-terminus of both KCNQ2 and KCNQ3. However, binding of Syntaxin to Helix A does not mediate Syntaxin's effect on the channel. We propose that the N-trminus of KCNQ2 plays a major role in the syntaxin's modulation since substitution of the proximal N-terminus of KCNQ2 with that of KCNQ3 abolished this effect.
Since the effect of syntaxin 1A is mediated through the N-terminus we assume that the N-C termini of the channel interact. To study this hypothesis we used florescence resonance energy transfer (FRET) experiments, single channel analysis and biochemical approaches using chimeric channels.
Together, our results point toward an allosteric modulation of KCNQ2 gating by Syntaxin 1A, facilitated by N-C termini interaction.

2316-Pos Board B302
Slowing of Instantaneous Inactivation Causes Macroscopic Current Decay in Kv7.1 Alanine Mutants
Vitya Vardanyan, Lijuan Ma, Olaf Pongs.
Alanine scanning mutagenesis in Kv7.1 S4-S5 linker and the pore region revealed mutant channels with fast current decay at permanent depolarizing pulses above 10mV. It has been previously shown that such a macroscopic inactivation of many Kv7.1 pore mutants occurs in parallel to instantaneous inactivation of Kv7.1 channel. We have chosen four mutant channels I268A, G269A, F339A and F340A with most pronounced current decays to investigate the molecular mechanism underling decay process. Inactivation of these channels could not be explained by intracellular Na+ block reported earlier for Kv7.1 wild type channel. The fast current decay kinetics significantly changed neither by varying the extracellular K+ concentration nor by replacement of the K+ with equimolar Rb+. Recovery from inactivation showed fast and slow components. The slow component was accelerated at high extracellular K+ conditions, whereas the fast component did not change significantly. Our data suggest that instantaneous inactivation of wild type Kv7.1 channel markedly slowed by above mentioned alanine mutations resulting in macroscopic decay of current during prolonged depolarization.

2317-Pos Board B303
Dynamic Modulation of Voltage-Dependent Kv7.2-7.3 Channel Opening by Voltage-Sensor Relaxation
Carlos A. Villalba-Galea, Junghoon Ha, I. Scott Ramsey.
S4-type voltage-sensor (VS) domains are common to voltage-gated ion channels (VGCs) and voltage-sensitive phosphatases (VSPs). In ciVSP, depolarization to +80 mV initially causes VS activation, generating rapid sensing charge (Q) movement. Sustained depolarization subsequently elicits a voltage-independent conformational rearrangement termed relaxation. Because the relaxed state is thermodynamically more stable than the activated state, relaxation causes a negative shift in the Q-V relation. Relaxation may be an intrinsic property of the VS domain because it is observed in the absence of the ciVSP catalytic domain. Since the VS and pore domains are coupled in VGCs, we wondered whether VS relaxation might alter voltage-dependent opening of an ion channel conductance (G). By analogy to ciVSP, we hypothesized that relaxation would shift the G-V relation toward negative potentials. In order to test this hypothesis, we used two-electrode voltage clamp to measure K+ currents in Xenopus oocytes expressing Kv7.2 and Kv7.3 mRNAs. As expected, prolonged depolarizations to +80 mV caused the steady-state G-V relation to shift toward negative potentials, indicating that VS relaxation alters the voltage-dependence of channel opening in heteromeric Kv7.2-7.3 channels. Furthermore, the magnitude of the shift in the midpoint of the G-V relation was found to depend on the duration of the +80 mV prepulse. Similar to ciVSP, the progressive shift in the G-V relation may be interpreted as an index of relaxation and used to measure the rates of entry into and recovery from the relaxed state. Our data therefore imply that the voltage dependence of VGC opening is likely to be dynamically modulated by conformational transitions that are intrinsic to the VS domain in a voltage-gated channel complex. VS relaxation may represent a widespread mechanism for regulating VGC function altering the relative positions of the Q-V and G-V relations.

2318-Pos Board B304
KCNQ1-R539W Mutation Substitutes Cholesterol for Phosphatidylinositol-4, 5-Bisphosphate in Channel Regulation
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Point mutation of nearby residues in ion channels can be associated with diametrically opposed clinical phenotypes despite the mutant channels exhibit similar biophysical characteristics. Hence a characterization of the channel structure/function at the amino-acid scale is required for better understanding of channel genotype/phenotype relationship. R555C and R539W KCNQ1 mutant channels are a good illustration of this idea: R555C mutation is associated with a fruste form of type 1 long QT syndrome, whereas R539W mutation is associated with sudden death. Puzzling enough, the genotype/phenotype relationship is difficult to understand because the mutated residues are in the same helix C module, they both concern arginine residues, both channels have the same biophysical properties, and the same sensitivity to short chain phosphatidylinositol-4,5-bisphosphate (PIP_2).
To better understand the genotype/phenotype relationship in the context of these mutations, we performed several tests in COS-7 cells expressing the WT or mutant channels and used tail-currents amplitudes as readout. We show that R539W is very peculiar: As opposed to WT and R555C channels, the R539W channel current is barely running down when available PIP_2 is decreased, either by wortmannin application in whole-cell, or by magnesium application in inside-out configuration. Consistent with that, the R539W channel is also insensitive to extracellular osmolarity, known to modulate the channel activity via PIP_2. These results suggest that KCNQ1-R539W mutation shortcuts PIP_2 in the channel open pore stabilization**.** Both structural model prediction and functional analysis implicate membrane cholesterol in this effect. Indeed, structural model prediction suggests that the introduced tryptophan in R539W interacts with cholesterol. Both cyclodextrin application on R539W and substitution of R539 by residues other than tryptophan restore channel rundown, consistent with the supposed tryptophan-cholesterol interaction. We conclude that the R539W/cholesterol interaction substitutes for R539/PIP_2 interaction in the channel open pore stabilization**.**

2319-Pos Board B305
N-Glycans Modulate hERG1A Window Current
Sarah A. Norring, Tara A. Schwetz, Andrew R. Ednie, **Eric S. Bennett**.
Voltage-gated K^+ channels are primarily responsible for the repolarization phases of neuronal, skeletal and cardiac muscle action potentials. Activity of the human *ether-a-go-go*-related gene 1 (hERG1) voltage-gated K^+ channel produces a K^+ current responsible for much of late phase II and phase III repolarization of the human cardiac action potential. HERG1A has two putative N-glycosylation sites located in the S5-S6 linker region, one of which is N-glycosylated. The aim of this study was to determine whether and how N-linked glycosylation modifies hERG1A channel function. Voltage-dependent gating of hERG1A were evaluated under conditions of full glycosylation, no sialylation, in the absence of complex N-glycans, and following the removal of the full N-glycosylation structure. The hERG1A steady-state activation relationships were shifted along the voltage axis by a significant, depolarizing ~9 mV under each condition of reduced glycosylation. Steady state channel availability curves were shifted by a much greater depolarizing 20-30 mV under conditions of reduced glycosylation. The depolarizing, non-uniform shifts in voltage-dependent steady state activation and inactivation caused a large rightward shift and an increase in the hERG1A window current. This suggests that reduced glycosylation caused an increase in the persistent hERG current that occurs at more depolarized potentials as observed. This increase and shift in window current would lead to increased hERG1A activity during the action potential, effectively increasing the rate of repolarization, and reducing AP duration, as predicted through *in silico* modeling of the ventricular AP. Overall, these data indicate a functional role for N-glycosylation in the modulation of hERG1A channel activity, suggesting that even small changes in channel N-glycosylation modulate hERG1A activity, and thereby likely impact the rate of action potential repolarization.

2320-Pos Board B306
Role of the Activation Gate in Determining the Extracellular Potassium Dependency of Block of HERG by Trapped Drugs
Kristeen Pareja, Barbara Olkiewicz, Kristopher Murphy, August Anderson, **Alan Miller**.
Block of the cardiac potassium channel HERG by a number of drugs has been shown to decrease with an increase in the extracellular potassium concentration. This dependency on extracellular potassium can be explained by at least two mechanisms: 1) destabilization of the drug by the permeant ion 2) differential binding to the inactivated state. We have previously shown that block of HERG by quinidine, a drug that is not trapped after channel deactivation, correlates better with the permeant ion than with inactivation, indicating that quinidine block is destabilized by the permeant ion.[1] We show here that block of HERG by terfenadine and bepridil, drugs shown to be trapped in the channel after channel deactivation[2], is not altered with an increase in the extracellular potassium concentration. Furthermore block by both terfenadine and bepridil of the HERG mutant D540K, which opens with both depolarization and