We were further able to optically monitor the light-induced membrane depolarizations on a millisecond timescale with the voltage-sensitive RH421. The fluorescence readout reflected the concentration-inhibition relationship of the hKv1.5 inhibitor DPO-1.
LIVC represents a solely optical technology with remote activation of the target voltage-gated ion channels simply by the delivery of a flash of blue light and simultaneous detection of their activity employing voltage-sensitive dyes. It combines the high-throughput of optical methods with the high-content of patch clamp concerning and possible repetitive stimulation. Proof of concept and results from assay development for voltage-gated sodium and calcium channels as well as for the hERG channel underline the potential for LIVC to evolve into a high-throughput, high reliability assay for voltage-gated ion channels in general.

914-Pos Board B793
Kv1.7 - Interactions with Protons and a blocking Conotoxin
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We have examined the interactions of protons and an inhibitory, poly-cationic conotoxin with the human voltage-gated potassium channel, hKv1.7. This channel differs from some members of the Kv1 sub family by having a titratable histidine residue near the N-terminal end of the putative pore-supporting P-helix, suggesting that intrinsic channel functions and pharmacology may depend on the pH of the external solution. Channels were expressed in HEK-293 cells and studied by whole-cell patch clamp. The voltage dependence of channel activation was evaluated using a tail-current protocol in which the voltage was stepped to -40 mV following a variable activating pre-pulse. Lowering of the pH of the external solution from 7.4 to 5.0 produced a positive shift in the half-activation of 36 ± 3 mV (n=4). The lowering of pH also dramatically decreased the ability of the conotoxin to inhibit currents through the channels. Thus, following the largest depolarization, at pH5.0 very little inhibition was observed at a toxin concentration near IC50 for pH7.4. The tail current in the presence of the conotoxin was near the value seen in the absence of the toxin for external pH of 7.4. Our observations are consistent with a two-fold action of the conotoxin. First, they suggest that the positively charged toxin binds close enough to the S4 segment of the voltage sensor to inhibit activation following a depolarizing voltage step. Second, the observed decrease in maximal conductance at pH 7.4 following addition of the toxin is consistent with a block of current through the open channels. Toxin binding appears to be inhibited by protonation of a residue on the external surface of the channel, perhaps the histidine residue near the N-terminal end of the pore helix.

915-Pos Board B794
Pore Block of KCNQ1 Channels by Zn^{2+} is Modulated by Ancillary Subunits
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The basolateral membrane K^+ channel, KCNQ1 (KvLQT1, Kv7.1), plays a critical role in anion secretion by gastrointestinal tissues because it establishes an electrical driving force for anion exit. In secretory tissues, KCNQ1 associates with the KCNE3 ancillary subunit to form a voltage-insensitive K^+ channel, whereas, in the heart, it associates with KCNE1 (minK) to form a voltage-gated channel. To demonstrate the role of KCNQ1 in anion secretion, we used forskolin to activate secretory short circuit current (Isc) across T84 human colonic cell monolayers. We demonstrate that addition of Zn^{2+} (5 mM), an inhibitor of intestinal secretion, to the serosal bath reduced Isc by 34%. Subsequent addition of the K^+ channel blocker, Ba^{2+} (5 mM), reduced the current to near zero. To determine the mechanisms of Zn^{2+} block, we expressed KCNQ1 in *Xenopus* oocytes and determined the effects of extracellular Zn^{2+} on current-voltage relationships. When KCNQ1 was co-expressed with KCNE1, K^+ currents were very slowly voltage-activated and Zn^{2+} had a small inhibitory effect (23% at Vm = +40 mV). Co-expression of KCNQ1 with KCNE3 resulted in K^+ currents that were constitutively active and voltage-insensitive, however, Zn^{2+} caused these currents to become slowly-activating and dramatically reduced (64%). The dose-inhibition curve for Zn^{2+} on KCNQ1 expressed with KCNE1 revealed a single binding site (EC_{50} = 2 μM). The curve for KCNQ1 with KCNE3 also revealed a single binding component, but with a much greater affinity (EC_{50} = 0.1 μM) than we previously determined for Ba^{2+} (EC_{50} = 100 μM). These results suggest that the site of regulation of the open or activated state of KCNQ1 by KCNE1 or KCNE3 involves the extracellular pore region. In addition, the ancillary subunits cause state-dependent differences in the block of KCNQ1 by Zn^{2+}.

916-Pos Board B795
Discovery of a Novel Activator of KCNQ1-KCNE1 K+ Channel Complexes
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KCNQ1 (Kv7.1) associates with the family of KCNE peptides to form complexes with diverse gating properties and pharmacological sensitivities. The varied gating properties of the different KCNQ1-KCNE complexes enables the same K^+ channel to function in both excitable and non excitable tissues. Small molecule activators would be valuable tools for examining the gating mechanisms of KCNQ1-KCNE complexes; however, there are very few known activators of KCNQ1 channels and most are ineffective on KCNQ1-KCNE complexes. Our lab has identified a simple boronic acid, phenylboronic acid (PBA), which potentiates KCNQ1-KCNE channel complexes in a voltage dependent manner. Activation by the boronic acid moiety has some specificity for the Kv7 family members (KCNQ1, KCNQ2/3, and KCNQ4) since PBA does not activate Shaker or hERG channels. We show potentiation of current is due to a slower rate of deactivation and a hyperpolarizing shift in the voltage sensitivity of the channel complex. Analysis of different-sized charge carriers revealed that PBA targets the permeation pathway of KCNQ1 channels. The discovery that PBA activates physiologically relevant KCNQ1-KCNE complexes makes it a useful and readily available tool to investigate the molecular mechanisms of KCNQ1-KCNE complex activation.

917-Pos Board B796
Using Inducible Expression Vector Technology To Create Stable Cell Lines Expressing KCNQ2/3, KCNQ4, And KCNQ3/5 Currents Suitable For Automated Electrophysiology Platforms
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The KCNQ (Kv7) family of voltage gated ion channels conduct a number of hyperpolarising currents in various tissue types, including the heteromultimeric KCNQ2/3 M-current found in sensory neurones. Cell lines constructed using constitutive expression vectors to stably transfect KCNQ2/3, KCNQ4, and KCNQ3/5 genes gave acceptable performance when using rubidium efflux methodology. However, expression levels within the cell population were found to be variable when assessed using conventional electrophysiology. Cell morphology changed during passage and the cell lines were unsuitable for automated electrophysiology recording. Using RheoSwitch™ inducible vector technology we have created new stable cell lines where the production of ion channel can be closely controlled by addition of an inducer agent. Putative clones were screened using IonWorks® Quattro™ recording in single hole PatchPlate™ mode. For each of the three cell lines, clones were identified displaying more than 60% of the cells having greater than 0.5nA of current. The performance of the clones in single hole mode was suitable for progression to Population Patch Clamp™ (PPC) mode recording. Each cell line displayed acceptable seal properties and current amplitudes, KCNQ2/3 26 ± 5 MΩ, 0.77 ± 0.19 nA (n=250); KCNQ4 112 ± 49 MΩ, 0.44 ± 0.07 nA (n=372) and KCNQ3/5 159 ± 44 MΩ, 0.84 ± 0.50 nA (n=124). In addition, each cell line each cell line displayed the appropriate pharmacology for regitabine, linopridine, XE991, TEA and bepridil. The cell lines are suitable for compound screening and selectivity profiling using automated and conventional electrophysiology.

918-Pos Board B797
The Dipeptidyl-peptidase-like-protein DPP6 Determines the Unitary Conductance of Neuronal Kv4.2 Channels
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The neuronal subthreshold-operating A-type K^+ current regulates electrical excitability, spike timing and synaptic integration and plasticity. The Kv4 channels underlying this current have been implicated in epilepsy, regulation of dopamine release, and pain plasticity. However, the unitary conductance (γ) of neuronal somatodendritic A-type K^+ channels composed of Kv4 pore-forming subunits is larger (~7.5 pS) than that of Kv4 channels expressed singly in heterologous cells (~4 pS). Here, we examined the putative novel contribution of the dipeptidyl-peptidase-like-protein-6 DPP6-S to the γ of native (cerebellar granule neuron, CGN) and reconstituted Kv4.2 channels. Co-expression of Kv4.2 proteins with DPP6-S was sufficient to match the γ of native CGN channels; and CGN Kv4 channels from *dpp6* knock-out mice yielded a γ indistinguishable from that of Kv4.2 channels expressed singly. Moreover, suggesting electrostatic interactions, charge neutralization mutations of two N-terminal

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.