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