lead optimization programs at Exelixis. Whole-cell recordings from an in-house developed cryopreserved CHO cell line heterologously expressing HERG channels were obtained with IonWorksQuattro™. Ion channel expression was unaltered for >10 months (>400 pA/well). Only population patch-clamp wells with seal test >25 MΩ and peak tail currents >100 pA were selected for analysis. Success rates consistently obtained were >90%. Pharmacological analysis, characterized by automated IC50 determinations, were compared with equivalent studies performed with a Giga-Ω seal manual patch-clamp system to evaluate the predictive competency of the automated instrumentation and then validate its effective impact on quantitative SAR analysis for the selection and prioritization of lead compounds, avoiding potential QT prolongation liabilities at early stages.

1652-Pos
Electrostatic Tuning of Cellular Excitability
Sara I. Börjesson[1], Teija Parkkari[2], Sven Hammarström[1], Fredrik Elinder[1].
[1]Linköping University, Linköping, Sweden, [2]Kuopio University, Kuopio, Finland.
Voltage-gated ion channels regulate the electric activity of excitable tissues like the heart and the brain. Therefore, treatment for conditions of disturbed excitability is often based on drugs that target ion channels. Traditional ion-channel drugs aim at plugging the ion-conducting pore. We instead propose a novel pharmacological mechanism for how to regulate channel activity by targeting the voltage sensor of voltage-gated K channels. By studying the effect of different free fatty acids and fatty acid derivatives we show that charged lipophilic substances can tune channel opening and consequently excitability by an electrostatic interaction with the channel. Polyunsaturated fatty acids shift the voltage dependence of activation of the Shaker K channel in hyperpolarizing direction. The negative carboxyl charge is crucial for the effect. A positively charged arachidonic acid derivative (arachidonyl amine) was synthesized and shown to instead shift the voltage dependence in depolarizing direction. Thus, the direction of the effect on the channel's voltage dependence is determined by the charge of the substance. Uncharged methyl esters of polyunsaturated fatty acids do not affect the voltage dependence. Computer simulations of membrane excitability demonstrate that small changes in the voltage dependence of Na and K channels have prominent impact on excitability and the tendency for repetitive firing. For instance, a shift in the voltage dependence of a K channel with −5 or +5 mV corresponds to a three-fold increase or decrease in K channel density, respectively. We suggest that electrostatic tuning of ion channel activity can be a new and powerful pharmacological approach to affect cellular excitability.

1653-Pos
Arming Antibodies for Subtype-Selective Photo-Inhibition of Voltage Gated Potassium Channels
Jon T. Sack[1,2], Nick Stephanopoulos[3], James S. Trimmer[1].
[1]University of California, Davis, CA, USA, [2]Institute for Design of Intelligent Drugs, Protean Research, Palo Alto, CA, USA, [3]University of California, Berkeley, CA, USA.
Establishing the molecular identity of native voltage-gated potassium (Kv) channels has been a particularly challenging problem. Mammalian Kv channels arise from a family of more than 40 genes and few inhibitors are selective for any one Kv subunit type. The identification of channel types underlying native ionic currents has been greatly aided by the availability of subtype specific inhibitors, but drugs of great selectivity have not yet been discovered for most Kv subunits. There exist, however, extensively characterized monoclonal antibodies against extracellular S1-S2 linker epitopes that exhibit clear specificity for Kv4.2, Kv2.1 or Kv1.1. Unfortunately, none of these antibodies have been found to inhibit channel currents.
A proven strategy for targeted inhibition of proteins is to label antibodies with chromophore ''warheads'' that induce oxidative damage to the target protein upon illumination. Photo-stimulation of certain chromophores leads to the generation of singlet oxygen, which has a 40 Å half-maximal radius of oxidative damage, suitable to oxidize the protein target when conjugated to an antibody. Porphyrins are amongst the most efficient photo-induced generators of singlet oxygen known, having greater extinction coefficients, lower sensitivity to photobleaching, and higher quantum yields for singlet oxygen than compounds, such as fluorescein, classically used for targeted photo-inhibition of proteins. We have synthesized a series of porphyrin derivatives that irreversibly inhibit Kv4.2 or Kv2.1 currents upon illumination. Covalent attachment of porphyrin to an anti-Kv4.2 antibody has resulted in selective inhibition of Kv4.2 at a 10 nM concentration. By attaching warheads to subtype-selective antibodies, we aim to find a serial solution to the problematic dearth of subunit-specific Kv inhibitors.

Anion Channels

1654-Pos
Cholesterol Depletion Facilitates Recovery from Hypotonic Cell Swelling of CHO
Gregory Kowalsky[1], Derek Beam[2], Frederick Sachs[3], Susan Hua[4], **Irena Levitan**[1].
[1]University of Illinois at Chicago, Chicago, IL, USA, [2]Analytical Instruments, Reichert, Bufallo, NY, USA, [3]University of Bufallo, Bufallo, NY, USA, [4]University of Bufallo, Bufallo, NY, USA.
The maintenance of cell volume homeostasis prevents pathological cell swelling that can lead to severe cellular dysfunction or death. A key step in maintaining cell volume in many cell types is activation of volume-regulated anion channels (VRAC). Our earlier studies showed that activity of VRAC is facilitated by a decrease in cellular cholesterol (Levitan et al 2000). These observations suggest that lowered cholesterol should also facilitate regulatory volume decrease (RVD), a process used by cells to recover from hypotonic swelling. The main constraint in testing this prediction, however, has been the lack of adequate methods to rapidly and reproducibly measure changes in cell volume of substrate-attached cells. In this study, we address this question using a novel microfluidic methodology from Reichert Inc. (CVC-7000), to measure cell volume response to hypotonic challenges (30% osmotic gradient) in real time. Cholesterol depletion facilitated the recovery from swelling via a more rapid onset of RVD (~130 s vs. 215 s in control and cholesterol depleted cells, respectively) and a higher degree of volume recovery after 10 min (41% ± 6% vs. 65% ± 6% in control and cholesterol depleted cells, respectively). In contrast, enriching the cells with cholesterol had no effect on RVD. These observations are consistent with our previous studies showing that while cholesterol depletion increases cell stiffness, cholesterol enrichment has no effect (Byfield et al 2004). These observations suggest that cholesterol depletion, and the consequent increase in cell stiffness, facilitates RVD by enhancing the activity of VRAC.

1655-Pos
Expression and Novel Function of Bestrophin-2 in Goblet Cells in Mammalian Colon
Kuai Yu.
Emory University, Atlanta, GA, USA.
Anion channels and transporters in the gastrointestinal epithelium play essential roles in fluid secretion and absorption and participate in regulating the pH and ionic composition of the gut luminal contents. Diarrheas produced by bacterial enterotoxins such as cholera and rotavirus are associated, respectively, with activation of two kinds of Cl- channels, the cystic fibrosis transmembrane conductance regulator (CFTR) and Ca2+-activated Cl- channels (CaCCs). Although the roles and mechanisms of CFTR are relatively well understood, CaCCs have remained enigmatic partly because their molecular identity has remained in question. Here we have investigated the role of bestrophin-2, a candidate CaCC protein, in colon using a mouse knockout model. Best2-/- knockout mice exhibit a greatly reduced amplitude of cholinergic (Ca2+)-stimulated anion secretion, consistent with Best2's potential role as a CaCC. However, unexpectedly, Best2 is expressed in the basolateral membrane of mucin-secreting colonic goblet cells and not in the apical membrane of colonocytes as predicted if it was a CaCC. Analysis of the cholinergically-stimulated anion secretion revealed that a large fraction of the current was carried by HCO3-, was unaffected by CFTR blockers, and was carried by Best2 channels. Whole cell patch clamp analysis of isolated colonocytes revealed two kinds of Ca2+-activated Cl- channels, currents with linear I-V relationships carried by Best2 and reduced in the knockout and outwardly-rectifying currents that resemble currents carried by TMEM16A, another candidate CaCC protein that is expressed on the apical membrane of surface colonocytes and is probably involved in Cl- absorption. These results provide a new perspective on cholinergic regulation of colonic secretion and may have relevance to colitis and inflammatory bowel disease, two diseases that exhibit defective anion transport. Further, they provide new insights into the functions of the enigmatic bestrophin family of anion channels.

1656-Pos
TMEM16A is Expressed in Vascular Tissues that Display Robust Calcium-Activated Chloride Currents
Alison J. Davies[1], **Abigail S. Forrest**[2], Thomas A. Jepps[1], William Sones[1], Rajesh Raghunathan[2], Iain A. Greenwood[1], Normand Leblanc[2].
[1]St Georges University of London, London, United Kingdom, [2]University of Nevada Reno, Reno, NV, USA.
Calcium activated chloride (Cl_{Ca}) channels are an important contractile mechanism in smooth muscle cells. Activation of these channels by calcium (Ca^{2+}) ions leads to Cl^- efflux and membrane depolarization. This depolarization then favors the activation of voltage-gated Ca^{2+} channels (e.g. L-type), providing a positive feed-back loop that allows for sustained contraction.