amino acids other than Gly does not result in the same phenotype as the epilepsy mutation, suggesting that a change in protein flexibility is involved in the effects of the mutation. Consistent with this result, altering the flexibility of the channel protein by changing viscosity of the intracellular solution also modulates Ca^{2+} sensitivity; and the epilepsy mutation reduces such modulation, possibly because it has already altered the flexibility. These results are consistent with a model that the peptide loop where the putative Ca^{2+} binding site and the epilepsy mutation are located acts as a spring-hinge for the conformational change of the AC region during channel opening; Ca^{2+} binding and the epilepsy mutation affect channel gating by altering the function of this spring-hinge.

1647-Pos

Regulation of Drk1 Channels by Carbon Monoxide and Carbon Monoxide-Releasing Molecule-2

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Carbon monoxide is a poisonous gas that is also syntethized in several tissues in the body, where it acts as a signaling molecule. CO has been shown to regulate ion channels such as voltage-dependent calcium channels (VDCC) and large-conductance calcium-activated potassium (BK) channels, a mechanism which is important for oxygen sensing in the carotid body. The Kv2.1 channel has been implicated with oxygen sensing in the pulmonary arteries and the ductus arteriosus, a process which may be regulated by carbon monoxide. We therefore investigated the effects of carbon monoxide and tricarbonyl dichlororuthenium dimmer (CORM-2), a widely used carbon monoxide releasing molecule, on the Kv2.1 channel. We found that, unlike with VDCC and BK channels, CORM-2 does not have the same effect as carbon monoxide: CO does not have an effect on the channel, while CORM-2 acts as an intracellular allosteric inhibitor of channel function. CORM-2 slows channel activation and deactivation kinetics by reducing the voltage-dependence of the rate constants. It also reduces overall open probability without affecting steady-state voltage-dependence. Manganese-decacarbonyl, another carbon-releasing molecule also does not have an effect on the Kv2.1, while ruthenium red seems to have biphasic effects, one mimicking the action of CORM-2 on the channel and the other representing voltage-dependent pore block, which suggests that CORM-2's actions on the Kv2.1 are independent from its CO-releasing properties.

1648-Pos

Mechanism of Kv1 Channel Redox Modulation by Kvβ

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The *Shaker* type voltage-dependent K^+ channels (Kv1) are expressed in a wide variety of cells and essential to regulating membrane potential and cellular excitability. All Kv1 channels assemble with cytoplasmic β subunits (Kvβ) to form a stable complex. Kvβ is an aldo-keto reductase that utilizes NADPH as a cofactor, and certain Kvβs have an N-terminal segment that blocks the channel by the N-type inactivation mechanism. The enzymatic activity and channel inactivation are functionally coupled: when the Kvβ1-bound NADPH is oxidized, the N-type inactivation is inhibited and, as a result, current increases. To understand the molecular basis of the coupling, we first focused on the N-terminal segment of Kvβ1 that induces channel inactivation. We have identified a stretch of amino acid residues from the N-terminus that are required for redox modulation, which we define as the Redox Regulation Sequence (RRS). Based on our studies, we found that it is likely that the RRS binds directly to the aldo-keto reductase core of Kvβ. To test this hypothesis, and to eventually construct a mechanism for redox modulation, we have started to identify regions on the AKR core that may serve as the ''receptor site'' for the RRS. Initial mutational studies have identified a candidate receptor site, and structural and biochemical studies will further examine how the physical interaction is achieved, and how the interaction is dependent on the redox state.

1649-Pos

Quantifying the Absolute Number of Voltage Gated EGFP Tagged Ion Channels by Fluorescence Intensity Measurements

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In the past, much work has been done to evaluate the gating charge, q, of single voltage gated K+ or Na+ channels. A figure of about 13 e is generally accepted for K+ channels. For Na+ channels, the figure of q estimated by various methods, is less well established and ranges from 5 to 15 e. Recently, we determined the gating charge of single Na+ channels indirectly to be about 6 e by applying our newly determined ratio of q K+ /q Na+ $= 2.5 \pm 0.4$ (Gamal El-Din et al. 2008) and assuming 13 e for q K+. Hereby we used the total fluorescence intensity of EGFP tagged channels expressed in X. laevis oocytes as a measure for the relative number of ion channels and correlated it to the total gating charge, Q.
Currently, we develop a more direct method to estimate the total number of ion channels per oocyte. Analysis of microscopic images of oocytes has been done according to Gamal El-Din et al. (2008). In addition, calibration of EGFP fluorescence intensity with EGFP solutions was done in a hemocytometer. Additional refinements for several correction factors have been tested to obtain reliable absolute numbers of ion channels: Especially the attenuation of the fluorescence from fluorophores at circumferential areas of the oocyte compared to those from frontal areas was taken into account. To obtain a measure of the attenuation factor we used oocytes homogeneously labeled with an extrinsically fluorescent dye and compared the total fluorescence intensity, extrapolated from cirumferential areas, with those extrapolated from frontal areas. Transfer of the attenuation factor of extrinsically labeled oocytes to the instrinsically EGFP labeled oocytes is being discussed and labeling profiles are shown.

1650-Pos

Voltage Clamping a Supported Bilayer

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Supported bilayer has many advantages over conventional black lipid bilayer. A supported bilayer is highly stable and can be made with a large surface area. Membrane proteins can be incorporated in very large numbers without disruption of the bilayer, thereby allowing robust macroscopic recordings. A very important advantage is that incorporated proteins are immobile. This lack of mobility is essential for the study of conformational changes with single molecule fluorescence. We are interested in studying gating charge movement and conformational changes in voltage dependent membrane proteins such as a voltage dependent K+ channel. For this purpose, we have developed an essentially electrostatic voltage clamp system for a supported bilayer that allows measurement of intramembrane transient currents but not DC currents. The supported bilayer is formed by liposome fusion on top of a semiconductor substrate that serves as the reference electrode. Electrolyte solution is present above the bilayer where an Ag/AgCl electrode serves as the active electrode. The electrode pair is connected to conventional voltage clamp electronics that imposes the voltage and measures the current. We verified supported bilayer formation by the decrease of the total capacitance. Furthermore, we have verified that a voltage is imposed across the bilayer by using voltage dependent fluorescent membrane probes. The electrolyte/supported bilayer/electrode system is essentially linear across a voltage range of −300 to +300 mV. We have seen that direct incorporation of the voltage dependent protein KvAP into the supported bilayer modifies the kinetics of the transient currents as well as the voltage dependence of charge movement. This method opens the possibility for studies of simultaneous gating charge movement electrically and voltage dependent conformational change spectroscopically in purified membrane proteins.
Support: NIH GM030376.

1651-Pos

Validation of Automated Patch-Clamp Instrumentation Competency for Herg Channel Liability Detection in Lead Optimization Programs

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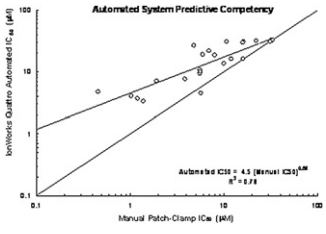
Avoidance of HERG Channel liability is an established regulatory requirement given its capabilities in predicting acquired Long-QT Syndrome, a cardiopathology which may lead to life threatening arrhythmias (Torsade de Points). The sensitivity and precision of patch-clamp electrophysiology, the gold standard in HERG safety pharmacology, have been expanded into the high throughput necessities of the drug discovery industry. This report presents results obtained in the early detection of possible HERG liabilities within



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
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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
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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
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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
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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
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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.