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.
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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