functionality [1]. To provide a more detailed understanding of the dynamic details of membrane-mediated perturbations of KcsA structure and function, we performed bulk and single-molecule fluorescence and electrophysiological assays of reassembled KcsA tetramers in neutrally charged DOPC and negatively charged DOPE/DOPG bilayers. Labeling of KcsA monomers was performed using the G56C KcsA mutant [2] which provides a solvent accessible reactive thiol for covalent coupling of fluorophores for FRET assays between neighboring monomers and fluorescently labeled membranes. The results suggest that structural rearrangement is predicated by KcsA-phospholipid headgroup binding which excludes non-negative phospholipids at the cytoplasmic TM2-membrane interface. Additionally, a limited number of inter-monomer structural perturbations are consistent with the data in the presence of negatively charged phospholipids, including a decrease in the angular relationship of KcsA monomers normal to the bilayer or decreased toroidal packing. The results of these assays shed light on the downstream structural effects of interactions of phospholipid headgroups on transmembrane proteins and how these correlate to function in a highly stable, model potassium channel.

1. FI Valiyaveetil, et al. Biochemistry (2002) 41:10771–10777.
2. M Iwamoto, et al. J. Biol. Chem. (2006) 38:28379–28386.

533-Pos Board B333

Semi Simultaneous Imaging of Ca^{2+} and Ca^{2+} Dependent Calmodulin Interactions by FRET

Mori X. Masayuki, Yuko Imai, Kyohei Itsuki, Ryuji Inoue.

Calcium dynamics and its linked molecular interactions contribute to a variety of biological responses, thus it is essential to exploit techniques for detecting both concurrently. Here we describe a new method to image Ca^{2+} and protein-protein interaction on semi-simultaneous base through combined application of Fura-2 fluorescence imaging and Fluorescence or F[[Unsupported Character - Örster Resonance Energy Transfer (FRET) with genetically encoded fluorescence proteins in single living cells. By applying Ca^{2+}-dependent corrections for Fura-2 fluorescence superposition on FRET images, we could semi-simultaneously analyze *in vivo* Fura-2 and FRET signals with high quantitative fidelity.

Furthermore, by producing gradual stepwise increases in $[Ca^{2+}]_i$ in HEK cells, we could obtain quantitative and synchronous dose-response relationships for $[Ca^{2+}]_i$ versus protein-protein interaction between Ca^{2+} binding protein 'calmodulin' (CaM) and its binding domain from an enzyme, which were fused with fluorophores CFP and YFP, respectively. The same approach applied to voltage-gated Na^+, Ca^{2+} and TRP channels revealed their distinct sensitivities to basal Ca^{2+} through CaM binding.

This semi-simultaneous imaging system could be applicable to the identification of Ca^{2+}-dependent protein interactions with further potential application to elucidating the interrelations between Ca^{2+} signals and cellular functions such as enzyme activities, signal transductions, and membrane protein regulations in various living cells.

534-Pos Board B334

KCNK3 (Task-1) Potassium Leak Channels Internalize in a PKC- and 14-3-3beta-Dependent Manner

Anatoli Lvov, Luke R. Gabriel, Ann R. Rittenhouse, Haley E. Melikian, William R. Kobertz.

Ion channels encoded by the human KCNK3 gene give rise to the pH-sensitive outwardly rectifier potassium leak current in a variety of cell types, where they play a decisive role in determination of the hyperpolarized resting membrane potential, controlling excitability and hastening repolarization. These tandem-pore domain channels are regulated by various agents and stimuli, including neurotransmitters, hormones, volatile anesthetics, oxidases and protein kinases. Several studies have shown that activation of protein kinase C (PKC) inhibits KCNK3 (Task-1) currents; however, the cellular mechanism underlying this inhibition has not been elucidated. Using a combination of electrophysiological, biochemical and imaging approaches, we have determined that PKC-activation induces KCNK3 channel internalization. Moreover, we have identified specific residues in the KCNK3 C-terminus that contribute, but are not solely required, for PKC-mediated internalization. Since it was previously shown that the interaction with phosphoserine binding protein 14-3-3beta promotes ER exit of KCNK3, we examined the role of 14-3-3beta on KCNK3 internalization. Using overexpression and shRNA knockdown experiments of 14-3-3beta, we show that PKC-dependent KCNK3 internalization is also crucially dependent on the cellular levels of 14-3-3beta protein. Our results reveal a novel mechanism of KCNK3 current regulation by channel internalization in a PKC- and 14-3-3beta-dependent manner.

535-Pos Board B335

Fluorinated General Anesthetics Modulate Shaker Potassium Channels at Clinically Relevant Concentrations

Maria I. Lioudyno, Michael T. Alkire, Yuri Sokolov, George K. Chandy, James E. Hall.

Recent studies demonstrated that selective intra-thalamic block of the Kv1.2 potassium channels rapidly reverses the unconsciousness of inhalational anesthesia (Alkire et al., 2009). Furthermore, intra-thalamic block of Kv1.3 with selective blocker, ShK, reverses the unconsciousness effect of sevoflurane. Both findings suggestthat Shaker-related potassium channels might be key targets for anesthetics. We tested this hypothesis using patch clamp recordings of potassium currents in cell lines stably expressing homomeric human Shaker family channels. We found that at low voltages, sub-clinical and clinical doses of sevoflurane irreversibly potentiate potassium current through Kv1.3>Kv1.2>Kv1.5>Kv1.1. channels, by increasing the peak current amplitude and accelerating the current activation kinetics. At higher voltages, clinical doses of sevoflurane inhibit Kv1.4>Kv1.5.>Kv1.3 and potentiate Kv1.2>Kv1.1. Sevoflurane had no effect on lipid bilayer conductance, suggesting direct interaction of the anesthetic with potassium channel subunits. Thus, Shaker potassium channel modulation may contribute to the clinical effects of general anesthesia. Supported by the Hillblom Foundation and NIH 1P01AG032131.

536-Pos Board B336

Dynamic Phosphorylation/Dephosphorylation of a Voltage-Gated K^+ Channel Controls Non- associative Learning

Diego Cotella, Federico Sesti.

The worm *C. elegans* exhibits habituation_an universally conserved form of non-associative learning_to repetitive mechanical stimulation. We have previously described a key player of habituation in *C. elegans*. This is a K^+ channel-kinase complex composed of the pore-forming subunit KHT-1 (73% homology to human Kv3.1), and the accessory subunit MPS-1 (55% homology to KCNE2), which shows kinase activity. Upon repetitive touching, the K^+ current carried by KHT-1 diminishes, as a result of MPS-1-mediated phosphorylation. At cellular level this mechanism induces neuronal adaptation, because the electrical activity of the touch neuron is altered and at behavioral level, habituation. Here, we characterize a novel player involved in this dynamic process, namely, a phosphatase termed ACP-2 which is encoded by the *f14e5.4* gene. Genetic, biochemical and electrophysiological evidence show that KHT-1, MPS-1 and ACP-2 form a stable, ternary complex *in vitro* and *in vivo* and that KHT-1 is de-phosphorylated when ACP-2 is present. Upon repetitive stimulation ACP-2 disengages from the complex allowing MPS-1 to phosphorylate the channel thus causing habituation. Taken together these data underscore a new mechanism of dynamic regulation of electrical signaling in the nervous system.

537-Pos Board B337

Cholesterol Sensitivity of Kir2.1 is Modulated by a Cytosolic Belt: Implications for Kir Gating

Avia Rosenhouse-Dantsker, Diomedes E. Logothetis, Irena Levitan.

Kir channels are important in setting the resting membrane potential and modulating membrane excitability. A common feature of Kir2 channels and several other ion channels that has emerged in recent years is that they are regulated by cholesterol, a major lipid component of the plasma membrane whose excess is associated with multiple pathological conditions. Yet, the mechanism by which cholesterol affects channel function is not clear.

We have recently shown that cholesterol sensitivity of Kir2 channels depends on several CD-loop residues. Interestingly, the importance of the CD loop to cholesterol sensitivity extends beyond the Kir2 family: corresponding mutations in the CD loop suppress cholesterol sensitivity of Kir3.4* channels.

Here we show that this cytosolic loop is part of a regulatory site that also includes residues in the G-loop, the N-terminus, and the linker between the C-terminus and the inner transmembrane helix. Together these residues form a cytosolic belt that surrounds the pore of the channel close to its interface with the transmembrane domain, and modulate cholesterol sensitivity of the channel.

Furthermore, we also show that the motion of residues in this cluster is correlated with movements of the most dynamic residues of the G-loop, the major cytosolic gate of Kir2.1. In contrast, no significant correlation was found between the dynamic G-loop residues and residues that were just outside the cytosolic belt. These results suggest that residues of the cytosolic belt are critical for channel gating and that cholesterol exerts its effects on channel function by affecting the gating machinery of Kir channels.