monitor the opening of single subunits while single channel current is recorded. Fluorescence intensity changes of FRET pairs or environment-sensitive dyes allow us to distinguish the movement of the four subunits (*Blunck et al., 2008*). We successfully imaged the diffusion of single channels in the bilayer using an EMCCD camera and now seek to correlate their fluorescence intensity, which is associated to a partial or a full opening, with the occurrence of subconductance levels.

This work is supported by CIHR MOP-81351, CRC 202965 & NSERC 327201DG; H.M. holds a student fellowship of FQRNT.

2771-Pos

Functional Incorporation of KcsA into Tethered Lipid Bilayer Membranes

Haw-Zan Goh[1], Matteo Broccio[1], Sidd Shenoy[1], Frank Heinrich[1,2], Joseph A Mindell[3], Mathias Lösche[1,2].
[1]Physics Dept, Carnegie Mellon University, Pittsburgh, PA, USA, [2]NIST Center for Neutron Research, National Institute of Standards and Technology, Gaithersburg, MD, USA, [3]NIDDK, National Institutes of Health, Bethesda, MD, USA.

Tethered lipid bilayer membranes (tBLMs) are solid-supported lipid bilayers separated by a ~ 2 nm thick hydrated layer from the solid interface. In comparison to cell membranes, they are simpler in their chemical composition and can therefore be quantitatively studied with a variety of experimental techniques. In comparison to free-standing or vesicle membranes, they are much more long-term stable. tBLMs can be formed by rapid solvent exchange,[1] which leads to highly electrically insulating, defect-free bilayers,[2] or by vesicle fusion, which results in membranes with higher residual conductance but makes protein reconstitution more straightforward. While we studied the structure and function of tBLMs produced by rapid solvent exchange extensively in the past,[2] we have more recently optimized vesicle fusion protocols for tBLM formation and observed with neutron reflectometry that the membranes resulting from the two preparation methods are very similar in their molecular structure. Fluorescence correlation spectroscopy shows that lipid-label diffusion is identical in both cases. In this work, we incorporate the tetrameric potassium-selective channel KcsA from *Streptomyces lividans* into tBLMs through vesicle fusion. KcsA is reconstituted into POPE/POPG lipid vesicles that are spread onto the solid support to form the membranes. The functionality of the reconstituted channel is confirmed by electrochemical impedance spectroscopy (EIS), where we observe that the resistance of tBLMs with KcsA is 3 to 10 times smaller than the resistance of neat tBLMs in the presence of K^+ ions, and with various blockers of the potassium channel.

Supported by the NIH (1P01AG032131) and the AHAF (A2008-307).

[1]Cornell, B.A, *et al.* 1997. *Nature* 387:580-583.
[2]Valincius, G., *et al.* 2008. *Biophys. J.* 95:4845-4861.

2772-Pos

Energetic Coupling Between Amino Acids in the pH-Sensing Region of the KcsA Channel

Ameer N. Thompson, Crina M. Nimigean.
Weill Cornell Graduate School of Medical Science, New York, NY, USA.

The bacterial potassium channel KcsA is gated by high concentrations of intracellular protons, allowing the channel to open at pH < 5.5. Replacing key ionizable residues from the N and C termini of KcsA with residues mimicking their protonated counterparts with respect to charge renders the channel open up to pH 9.0 (Thompson et al., 2008). We proposed that these residues function as the proton-binding sites. At neutral pH they form a complex network of inter- and intrasubunit salt bridges and hydrogen bonds near the bundle crossing, stabilizing the closed state. At acidic pH, these residues change their ionization state, thereby disrupting this network, favoring channel opening. To gain insight into the interactions that govern channel opening, we performed a thermodynamic analysis of the residues in the pH-sensing region. Individual mutations of most residues in this region result in modest shifts in the pH dependence of channel opening. However, pair-wise mutations of a subset of these amino acids show a large shift on the pH dependence of the channel opening suggesting these amino acids interact to open the channel with protons.

2773-Pos

Membrane Trafficking Controls K2P1/TWIK1 Channel Expression at the Cell Surface

Sylvain F. Feliciangeli[1], Said Bendahhou[2], Magalie P. Tardy[1], Guillaume Sandoz[1], Franck C. Chatelain[1], Markus Reichold[3], Richard Warth[3], Jacques Barhanin[4], Florian Lesage[1].
[1]CNRS UMR 6097, Valbonne, France, [2]CNRS FRE3093, NICE, France, [3]Institute of Anatomy, University of Regensburg, Regensburg, Germany, [4]CNRS FRE 3093, Nice, France.

Two-P-domain potassium (K2P) channels produce background conductances involved in neuronal excitability and cell volume regulation. In contrast with other K2P channels, little is known about TWIK1 (K2P1), despite the fact that it has been the first K2P channel cloned and expressed (Lesage et al, EMBO J. 1996, 15, 1004-1011). Functional studies on TWIK1 have been impeded by the fact that it produces only modest current upon heterologous expression in Xenopus oocytes, and that so far, no currents similar to TWIK1 have been reported in native cells. It has been proposed that K2P1 was present at the cell surface but silenced by conjugation of a SUMO peptide to an unconventional sumoylation site (Rajan et al, Cell. 2005, 121, 37-47). However, we did not observe any quantitative sumoylation of TWIK1 in vivo or even in vitro. Also, we have shown that inactivation of the putative sumoylation by a conservative lys to arg mutation was without effect on the level of TWIK1 current (Feliciangeli et al, Cell. 2007, 130, 563-569). We now provide new evidence demonstrating that the lack of measurable current upon TWIK1 expression in mammalian cells is caused by its active endocytosis from cell surface and retention in intracellular recycling endosomes. Inactivation by point mutation of an unusual endocytosis signal sequence produces a mutated TWIK1 channel that is expressed at the cell surface and produces measurable currents in all the cell types that have been tested.

2774-Pos

Helix C Regulates Surface Expression of KCNQ2 (kv7.2) Channels

Juncal Fernandez Orth[1], Paloma Aivar[1], Meritxell Rourai Ferrer[1], Juan Camilo Gómez Posada[1], Teresa Zamalloa[1], Alessandro Alaimo[1], Pilar Areso[2], Álvaro Villarroel Muñoz[1].
[1]Unidad de Biofisica CSIC-UPV/EHU, Leioa, Spain, [2]Departamento de Farmacología UPV/EHU, Leioa, Spain.

KCNQ2 (Kv7.2) subunits is one of the main subunits that give rise to the M-current, which play a prominent role in the control of neuronal excitability. Little is known regarding how the density of KCNQ2 channels at the plasma membrane is controlled. We have used the Tac membrane protein (also known as CD25/interleukin-2 receptor) as a reporter for the identification of critical traffic determinants. Fusion of helix C to Tac prevented trafficking to the plasma membrane. Within helix C, we identified the sequence RIK as a key player in the process. After deletion or neutralization to AIA or NIN, the surface expression increased, suggesting that this motive may function as a retention/retrieval signal. A natural existing mutant at this site, R553Q, is associated with neonatal epilepsy (BFNC), reflecting an important role of this sequence on KCNQ channel physiology.

2775-Pos

Lack of Clinically Important hERG Channel Block by the Antipsychotics Tiapride and Sulpiride

So-Young Lee[1], Hee-Kyung Hong[2], **Su-Hyun Jo**[2].
[1]Department of Life Science, Pohang University of Science and Technology, Pohang, Republic of Korea, [2]Department of Physiology, Kangwon Natiional University School of Medicine, Chuncheon, Republic of Korea.

The human *ether-a-go-go*-related gene (*hERG*) channel is important for repolarization in human myocardium and is a common target for drugs that prolong the QT interval. We studied the effects of two antipsychotics, tiapride and sulpiride on hERG channels expressed in *Xenopus* oocytes and also in the delayed rectifier K^+ current of guinea pig cardiomyocytes. The amplitude of the hERG outward currents measured at the end of the pulse showed no concentration-dependent change with increasing either tiapride or sulpiride concentration (3-300 μM). Also, the amplitude of hERG tail currents did not show concentration-dependent changes with increasing either tiapride or sulpiride concentration (3-300 μM). However, our findings showed that tiapride increased the values of the potential for half-maximal activation ($V_{1/2}$) at 10 - 300 μM, on the contrary, sulpiride increased the maximum conductance (G_{max}) at 3, 10, 100 μM. In guinea pig ventricular myocytes, bath applications of 100 and 500 μM tiapride at 36°C blocked rapidly activating delayed rectifier K^+ current (I_{Kr}) by 40.3% and 70.0%, respectively. Also, sulpiride at 100 and 500 μM blocked I_{Kr} by 38.9% and 76.5%, respectively, but tiapride and sulpiride at the concentrations did not significantly block slowly activating delayed rectifier K^+ current (I_{Ks}). Our findings suggest that the concentrations of the antipsychotics required to evoke a 50% inhibition of the I_{Kr} were well above reported therapeutic plasma concentrations of free and total compound. None of tiapride and sulpiride was a potent blocker of the hERG channel.

2776-Pos

Biologically Closed Electrical Circuits and Voltage Gated Ion Channels in Plants

Alexander G. Volkov[1], Justin C. Foster[1], Talitha A. Ashby[1], Vladislav S. Markin[2].
[1]Oakwood University, Huntsville, AL, USA, [2]University of Texas, Southwestern Medical Center, Dallas, TX, USA.