Temperature dependent steady state NOE experiments and NMR linewidth measurements indicate increased molecular motion in the EF-hand consistent with a proposed role for PC2-EF as a Ca2+-sensitive regulator. Structure-based sequence conservation analysis reveals a conserved hydrophobic pocket in this region, where PC2-EF may mediate Ca2+-dependent protein interactions. Using results of our structural studies we have examined the role of the EF-hand and coiled coil on PC2 channel function in single-channel lipid bilayers. Our results suggest that the coiled coil regulates PC2 by serving as an homo-oligomerization motif, whereas the EF-hand modulates the Ca2+-dependence of PC2 channel activity. Based on our results we propose a mechanism of regulation of the Ca2+-dependence of PC2 channel activity by PC2-EF.

2751-Pos

New Channels in the Outer Mitochondrial Membrane

Vivien Krüger[1], Michael Meinecke[2], Lars Becker[3], Chris Meisinger[4], Nikolaus Pfanner[4], Richard Wagner[1].
[1]University of Osnabrueck, Osnabrueck, Germany, [2]LMB Cambridge, Cambridge, United Kingdom, [3]University of Stanford, Stanford, CA, USA, [4]University of Freiburg, Freiburg, Germany.

Mitochondria are the "power stations" of eukaryotic cells. Beside this they play essential roles for the metabolism and physiology of cells and are a central point of apoptosis regulation. Mitochondria are also involved in calcium homeostasis. Due to endosymbiontic engulfment mitochondria are surrounded by two membranes. While the regulation of the metabolite flux across the inner membrane (IMM) is extensively characterised, it has been generally assumed that the outer membrane (OMM) functions only as a barrier for molecules larger than 3 kDa. But recent studies demonstrate that the metabolite flux between the cytosol and the different compartments of mitochondria is regulated at the level of the outer membrane.

Three pore forming proteins are up to now known in the outer membrane. Two of them are essential and involved in protein transport and insertion into OMM. These are Tom40 and Sam50/Tob55. The third one is the non-essential metabolite pore VDAC (voltage-dependent anion channel). The none lethal phenotype of VDAC knockouts discloses that it is the sole metabolite conducting pore in the OMM and the presence of other non-identified channels in the OMM is very likely.

The OMM proteome contains more than 112 proteins and only for less than 10 % of them the function is known. By electrophysiological screening of highly pure $OMM_{vdac\ \Delta}$ vesicles it was possible to identify at least four distinct membrane pores. In a first bioinformatical attempt using specific parameters like the isoelectric point or second structure prediction programs we identified eight potential channel candidate proteins.

2752-Pos

Evidence for Lateral Budding and Voltage Dependence of a Proteo-Lipid Channel

Debra Datskovskiy, **Meenu N. Perera**, Megan Miles, Toan Nguyen, Marco Colombini.
University of Maryland, College Park, MD, USA.

The pro-apoptotic protein, Bax, and the sphingolipid, ceramide, can individually form channels in phospholipid membranes. When combined, they permeabilize membranes in a synergistic way, indicating the formation of a combined channel structure. Nanomolar quantities of $LaCl_3$ disassemble ceramide channels completely but 10 micromolar $LaCl_3$ is needed to convert one large Bax-ceramide channel into a population of virtually identical channels. These channels exhibit voltage-dependent closure or disassembly. Some of the channels can be reassembled by reducing the voltage or applying an opposite potential but cycles of voltage-dependent closure and reopening quickly result in loss of conductance. There are indications that the transformation of the one large channel into a population of small channels occurs by lateral budding in the plane of the membrane. Over 100 such small channels were formed in one experiment and the application of an elevated potential resulted in a long staircase of virtually identical conductance decrements. These results open a window into phenomenology that, to our knowledge, has not been described previously.
(Supported by NSF grant: MCB-0641208)

2753-Pos

A Kinetic Model of Ion Channel Electrophysiology: Incorporating Bilayer-Mediated Effects of Agonists and Anesthetics on Protein Conformational Transitions

Robert S. Cantor, Kathryn S. Twyman, Daniel J. Albershardt.
Dartmouth College, Hanover, NH, USA.

The time- and concentration dependence of agonist-induced ion currents through postsynaptic receptors is often remarkably complex, involving desensitization and deactivation on multiple time scales, as is the modulation of these currents by other solutes such as anesthetics. Traditional kinetic models have involved agonist binding and conformational transitions among a very large manifold of protein conformational states engineered to reproduce the complexity of a particular set of electrophysiological results. However, independent experimental evidence for the hypothetical additional conformational states (beyond the minimal set of resting, conducting and desensitized) is essentially nonexistent, nor is there any model-independent way of estimating the values of the associated kinetic parameters. We propose an alternative model that includes only these three essential states while additionally incorporating the adsorption of agonist and nonbinding compounds such as anesthetics to the bilayer in which these intrinsic membrane proteins are embedded [R. S. Cantor et al., *Soft Matter*, 2009, **5**, 3266]. Solute adsorption alters bilayer physical properties, which in turn distorts the protein conformational free energy landscape, and thus alters the rate constants of protein conformational transitions. The complexity of the predicted ion currents - often well approximated as sums of exponentials - then arises from the time-dependence of solute adsorption, resulting in strongly time-dependent transition rate ''constants''. If only nonbinding solutes are present, the model simplifies considerably. For this special case, best fits of predicted current traces with respect to a small set of parameters are in excellent agreement with fast-perfusion electrophysiological studies of recombinant $GABA_A$ receptors [R. Haseneder et al., *Eur. J. Pharm.*, 2002, **451**, 43] in which currents are induced in the absence of agonist by a broad range of supraclinical concentrations of isoflurane and sevoflurane.

2754-Pos

KChiP2 Stabilizes Kv4 Protein Expression and Cell Surface Retention to Control Cardiac Ito Channel Densities

Nicholas C. Foeger[1], Céline Marionneau[2], Jeanne M. Nerbonne[1].
[1]Washington University in St. Louis, St. Louis, MO, USA, [2]Institut du Thorax, Nantes, France.

The myocardial transient outward current (I_{to}) is encoded by voltage-gated potassium (Kv) channel α-subunits of the Kv4 subfamily, together with the cytosolic accessory subunit, KChIP2. Targeted deletion of KChIP2 (KChIP2-/-) or Kv4.2 (Kv4.2-/-) eliminates I_{to} in adult mouse ventricular myocytes. Heterologous co-expression with KChIP2 increases Kv4.2 current densities and results in a relative shift in Kv4.2 from a perinuclear localization to the cell surface, leading to the suggestion that KChIP2 alleviates retention of assembled Kv4 channels in the endoplasmic reticulum (ER) and promotes forward trafficking. To explore these hypotheses, a putative RXR-type ER-retention motif at residues 35-RKR-37 in Kv4.2 was mutated (Kv4.2AAA), and the functional consequences of this construct on Kv4.2 expression in human embryonic kidney-293 (HEK) cells were explored. Mean ± SEM peak Kv4.2 current densities in cells expressing Kv4.2AAA (316 ± 50 pA/pF) were significantly ($p=0.025$) higher than in cells expressing wild type Kv4.2 (174 ± 20 pA/pF). Surprisingly, however, adenoviral expression of Kv4.2AAA in Kv4.2-/- myocytes resulted in peak Kv current densities (86 ± 9 pA/pF) that were not significantly different from the peak Kv currents (72 ± 9 pA/pF) in Kv4.2-/- cells infected with wild type Kv4.2. Heterologous expression of a charge-conservative mutant, Kv4.2KKK, in which arginines 35 and 37 were mutated to lysines (Kv4.2KKK), resulted in Kv4.2 currents (172 ± 25 pA/pF) that were indistinguishable from wild type currents, demonstrating that the presence of charged residues in the Kv4.2 N-terminus affects channel gating, not channel trafficking. Biochemical studies revealed no differences in the surface expression of the Kv4.2AAA mutant and wild type Kv4.2, and the surface expression of both constructs was increased dramatically upon co-expression of KChIP2. The results of further biochemical studies suggest that KChIP2 functions to increase the retention of Kv4.2 channels at the cell surface.

2755-Pos

The Na^+-Activated Potassium Channel Slack Shares a Similar Na^+ Coordination Site with Kir3 Channels

Zhe Zhang[1], Avia Rosenhouse-Dantsker[2], Qiongyao Tang[1], Sergei Noskov[3], Diomedes E. Logothetis[1].
[1]Department of Physiology and Biophysics, School of Medicine, Virginia Commonwealth University, Richmond, VA, USA, [2]Department of Medicine, University of Illinois at Chicago, Chicago, IL, USA, [3]Institute of Biocomplexity and Informatics, University of Calgary, Calgary, AB, Canada.

Characteristics of Na^+ activated potassium channel (Slack or Slo2.2) currents, including high conductance, rundown, regulation by Na^+, Cl^- and phosphorylation have long been reported but underlying mechanisms remain unknown. Here we report identification of a sodium regulatory site in the RCK2 domain of Slack channels by screening the C-terminus with the conserved sodium coordination motif of Kir channels. While the charge preserving D818E mutation exhibited similar Na^+ sensitivity as the wild-type Slack channel, both

a neutralization mutant D818N and a charge reversal D818R mutant within this site dramatically decreased Na^+ sensitivity. Thus, D818 is engaged in an important electrostatic interaction with Na^+. Similarly, the H823N mutant within this site also greatly decreased Na^+ sensitivity of Slack channels. Simulations of the Slack RCK2 domain based on the crystallized structure of a prokaryotic RCK domain structure (Jiang et al., 2001, Neuron 29:593) provided a model of the Na^+ coordination site in Slack channels. Moreover, simulations of the Na^+ coordination site in Slo2.2 channels predicted a 5~7 fold selectivity for Na^+ over Li^+ that were confirmed by electrophysiological data. Our results suggest that the Slack channel shares a similar Na^+ regulatory mechanism with Kir channels but with important differences, such as an intricate coupling mechanism to Cl^- co-regulation and possibly additional Na^+ sensitive sites.

2756-Pos

Oxidation of K^+ Channels Leads to Progressive Decline in Sensory Function during Ageing

Federico Sesti, Shi-qing Cai.
UMDNJ, Piscataway, NJ, USA.

Reactive oxygen species (ROS) play an important role in the progressive neuronal function loss that is part of both the normal ageing process and neurodegenerative disease. A central question is whether voltage-gated K^+ (Kv) channels, which are key regulators of neuronal excitability, are physiological targets of ROS and whether these interactions have a role in the mechanisms underlying age-related neurodegeneration. Here, we show that oxidation of K^+ channel KVS-1 during ageing causes sensory function loss in *Caenorhabditis elegans,* and that protection of this channel from oxidation preserves neuronal function.- Thus, chemotaxis to biotin and lysine, a function controlled by KVS-1, was significantly impaired (70%) in normal or wild-type young worms exposed to chloramine-T (CHT) or hydrogen peroxide (H_2O_2), but only moderately affected (35%) in worms harboring an oxidation-reduction (redox)-resistant KVS-1 mutant (C113S). In ageing C113S worms, the effects of free radical accumulation were significantly attenuated (40% loss-of-function) compared to wild-type (75%). Electrophysiological analyses showed that both ROS accumulation during ageing, and acute exposure to oxidizing agents, acted primarily to modify native KVS-1 channels expressed in the ASER neuron (which mediates chemotaxis) and as a consequence altered the excitability of neurons harboring wild-type but not C113S KVS-1. Together, these findings establish a pivotal role for ROS-mediated oxidation of voltage-gated K^+ channels in sensorial decline during ageing.

2757-Pos

Regulation of the Cardiac I_{ks} Channel Complex by Ubiquitylation and De-Ubiquitylation

Katarzyna Krzystanek[1], Morten Grunnet[1], Søren Peter Olesen[1], Hugues Abriel[2], Thomas Jespersen[1].
[1]University of Copenhagen, Copenhagen N, Denmark, [2]University of Bern, Bern, Switzerland.

KCNQ1 and its β-subunit KCNE1 form the delayed rectifier potassium current I_{Ks}, playing an important role in repolarisation of the cardiac tissue and in water and salt transport across epithelial tissues. In the heart I_{Ks} is partly responsible for terminating the cardiac action potential. Malfunctions in this channel can result in arrhythmias leading to cardiac arrest.

In heart physiology proper function and regulation of I_{Ks} current is essential. It has been reported that one of the mechanisms controlling the membrane density of KCNQ1 channels is mediated by ubiquitylation. I_{Ks} was shown to be down-regulated by Nedd4/Nedd4-like ubiquitin-protein ligases and this interaction was dependent on the PY-motif on the C-terminal of KCNQ1. Recently it was also discovered that epithelial sodium channel ENaC is regulated by the reverse process - de-ubiquitylation, mediated by an enzyme USP2 (ubiquitin-specific protease 2), which is one of the best described de-ubiquitylases. Therefore the aim of the work was to investigate whether a similar mechanism is valid for KCNQ1/E1 channel complex.

The effect of USP2-mediated de-ubiquitylation on I_{Ks} channel was investigated using electrophysiology and biochemistry. We observed that when KCNQ1/E1 was co-expressed with USP2-45 or USP2-69 isoform and Nedd4-2 in oocytes, USP2 counteracted the Nedd4-2-specific down-regulation of I_{Ks}. It resulted in a rescue of the current amplitude, which was then comparable to the one of I_{Ks} expressed alone. Biochemical studies of transfected HEK293 cells confirmed this observation as both total and surface expressed KCNQ1 protein was more abundant when co-expressed with USP2-45/-69 and Nedd4-2 as compared to Nedd4-2 alone. Co-immunoprecipitation assay suggested that USP2 can bind to KCNQ1 independently of the PY-motif and the presence of Nedd4-2.

These results point towards an interplay between ubiquitylating enzymes and de-ubiquitylases acting on I_{Ks} channel complex *in vitro*.

2758-Pos

Mechanism for Strict Regulation of Certain K+ Channels by Small, Fast Changes in Cell Volume

Maria de los Angeles Tejada[1], Kathleen Stolpe[1], Sofia Hammami[2], Niels J. Willumsen[2], Asser N. Poulsen[1], **Dan A. Klaerke**[1].
[1]University of Copenhagen, Frederiksberg, Denmark, [2]University of Copenhagen, Copenhagen, Denmark.

A number of physiological processes, such as salt and water transport, neuronal activity, migration and apoptosis, involve changes in cell volume. The response of the cells to such challenges often is a regulatory volume decrease (RVD), a mechanism which involves activation of K^+ channels. However, so far it has not been entirely clear which types of K^+ channels should be considered sensitive to cell volume changes and, in particular, the mechanism for regulation has been obscure. To address this issue, we have co-expressed a number of K^+ channels with aquaporins in *Xenopus laevis* oocytes and subsequently induced changes in cell voume by exposure to hypo- or hypertonic media. In all cases, the results are very clear; some K^+ channels (e.g. KCNQ1 and 4, Kir4.1/5.1, Ca^{2+}-activated IK and SK) are strictly regulated by small, fast changes in cell volume (approx. 5 %), whereas others are not (e.g. KCNQ2/3, Slo1 (BK) and Slo2.2 (slack)). Most recently, we have shown that the high-conductance slick channel (Slo 2.1) is dramatically stimulated (to 196 % of control) by cell swelling and inhibited (to 44 % of control) by a decrease in cell volume. Our results show that the mechanism responsible for the strict regulation of certain K^+ channels by small, fast changes in cell volume, in some cases, involve the cytoskeleton.In contrast, cellular release of ATP is not involved, and the regulation is not mediated by membrane stretch. Our recent finding, that the high conductance slick channel is highly cell volume sensitive, will allow for further investigations at a single channel level.

2759-Pos

Fluorinated General Anesthetics Modulate Kv1.3 Potassium Channels and Interact With β-Amyloid Peptide: Is there a Link?

Maria I. Lioudyno, Michael T. Alkire, Virginia Liu, Philip R. Dennison, Charles G. Glabe, James E. Hall.
University of California, Irvine, Irvine, CA, USA.

There is growing evidence that, in some cases, commonly used general anesthetics may cause long-term molecular changes reminiscent of those observed in the Alzheimer's diseased brain. We investigated the effects of the anesthetic sevoflurane on the voltage-gated potassium channel Kv1.3. In the central nervous system, Kv1.3 channels are present in olfactory regions and in the dentate gyrus of the hippocampus, areas implicated in AD pathology. The expression of Kv1.3 is also up-regulated in activated microglia, suggesting its possible role in microglial response to β-amyloid peptide. Using whole-cell patch clamp recording from L929 cells stably expressing Kv1.3, we found that sevoflurane modulates biophysical properties of the Kv1.3 channel. At clinically relevant concentrations, sevoflurane biphasically alters peak current amplitude, irreversibly facilitating the current at lower voltages (EC50 ~ 1/2 MAC) and reversibly inhibiting it at higher voltages (IC50 ~ 1 MAC). The kinetics of the Kv1.3 current were also changed in a voltage- and dose-dependent manner. The time constants of both current activation and the slow C-type inactivation were significanly decreased, whereas current deactivation was slower at low voltages but faster at higher voltages in the presence of sevoflurane. Sevoflurane slightly increased the voltage sensitivity of Kv1.3 conductance at a clinically relevant dose. The effects of sevoflurane resemble the previously-reported effects of the exogenous β-amyloid oligomers on the same channel. Using ^{19}F NMR, we found that, in the test tube, sevoflurane interacts with β-amyloid peptide and forms stable complexes. Furthermore, dot blot immunochemistry revealed that sevoflurane appears to facilitate the rate of cytotoxic β-amyloid oligomer formation.Thus, modulation of Kv1.3 channels by sevoflurane and its interaction with β-amyloid peptide might both enhance the progression of Alzheimer's disease. *Supported by the Hillblom Foundation and NIH 1P01AG032131.*

2760-Pos

Modulation of Plant Slow Vacuolar (sv) Channel by Flavonoid Naringenin

Paul Vijay K. Gutla[1,2], Armando Carpaneto[1], Alex Costa[2], Fiorella Lo Schiavo[2], Franco Gambale[1].
[1]Istituto di Biofisica, CNR, Genova, Italy, [2]Dipartimento di Biologia, Università degli Studi di Padova, Padova, Italy.

The Slow Vacuolar (SV) channel is one of the most extensively studied channel present in plant vacuoles. Features of the SV channel are the slow activation, outward rectification at elevated cytoplasmic Ca^{2+} concentration and selectivity for both monovalent and divalent cations. It's well known that SV currents recorded in a typical patch-clamp experiment require unphysiologically high cytosolic and low vacuolar calcium concentrations for full activation. We aim at identifying endogenous plant substances which