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.

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2771-Pos

Functional Incorporation of KcsA into Tethered Lipid Bilayer Membranes

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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.

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[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

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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

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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

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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

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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

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The Venus flytrap is a marvel of plant electrical, mechanical and biochemical engineering. The rapid closure of the Venus flytrap upper leaf in about 0.1 s is one of the fastest movements in the plant kingdom. We found earlier that the electrical stimulus between a midrib and a lobe closes the Venus flytrap upper leaf without mechanical stimulation of trigger hairs. The Venus flytrap can accumulate small subthreshold charges, and when the threshold value is reached, the trap closes. Thigmonastic movements in the sensitive plant Mimosa pudica L., associated with fast responses to environmental stimuli, appear to be regulated through electrical and chemical signal transductions. The thigmonastic responses of Mimosa pudica can be considered in three stages: stimulus perception, electrical signal transmission, and induction of mechanical, hydrodynamical and biochemical responses. We investigated the mechanical movements of the pinnae and petioles in Mimosa pudica induced by the electrical stimulation of a pulvinus, petiole, secondary pulvinus, or pinna by low electrical voltage and charge. Both voltage and electrical charge are responsible for the electro stimulated closing of a leaf. The mechanism behind closing the leaf in Mimosa pudica is discussed. The hydroelastic curvature mechanism closely describes the kinetics of Mimosa pudica leaf movements.

2777-Pos

Isoform- and Species-Specific Proteolysis of Cardiac Pacemaker Channels

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Proteolysis of cardiac pacemaker channels affects biophysical properties of functional channels. Hyperpolarization-activated channels HCN2 and HCN4 can form homomeric or heteromeric functional pacemaker channels in cardiac ventricles. Employing Western blot and immunoprecipitation techniques with antibodies against N- or C- terminus of HCN2 or HCN4, respectively, we investigated protein expression patterns of endogenous HCN2 and HCN4 in cardiac ventricles of small (mouse, rat) and large (sheep, canine) animals and human. Using an antibody against N-terminus of HCN2, more full length protein at 100kD and less cleaved bands around 50kD were detected in small than in large animals. An additional cleaved band around 60kD was exclusively expressed in human. HCN2 C-terminal antibody could not detect any full length protein in all species tested. A 75kD cleaved band was detected in mice, rat, canine and substantially higher in sheep heart ventricles. A 60kD band was observed in human only. Using an N-terminal HCN4 antibody, the full length protein signals (at 160kD) were present in sheep and canine only. The cleaved bands near 100kD predominated in small animals but absent in large animals. With a C-terminal HCN4 antibody, the full length protein was observed in mice, barely detectable in rat, and clearly present in sheep, canine and human. A cleaved band around 100kD predominated in all animals. A minor cleaved band around 50kD appeared in all tested species except human. Overall, there was less HCN2 and more HCN4 proteolysis in small than in large animal cardiac ventricles. Endogenous myocardial HCN2 and HCN4 underwent intensive proteolysis at both N- and C- termini in an isoform- and species-specific pattern. In conclusion, results obtained from HCN2 and HCN4 protein expression in small animals may not be directly applied to large ones including human.

2778-Pos

Mood Stabilizers Activate TREK-1, but not TREK-2

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Earlier studies have reported that channel activity of TREK-1 and TREK-2, members of two-pore domain K^+ (K_{2P}) channel family, are inhibited by antidepressants and antipsychotics such as fluoxetine, norfluoxetine, paroxetine, and chlorpromazine. This study was carried out to investigate the effect of mood stabilizers which are commonly used for treatment of bipolar disorder on TREK-1 and TREK-2 channels. In HEK-293A cells transfected with human TREK-1 or TREK-2, LiCl, gabapentin, valproic acid, and carbamazepin increased TREK-1 currents by $43 \pm 14\%$, $25 \pm 11\%$, $28 \pm 12\%$, and $82 \pm 11\%$, respectively, whereas these mood stabilizers have no effect on TREK-2 channel activity. The application of lamotrigine, a mood stabilizer inclined toward depression, failed to change TREK-1 channel activity. These results suggest that TREK-1 could be therapeutic potential target for treatment of bipolar disorder as well as depression.

2779-Pos

Color Shifted Channelrhodopsins- Towards Red Light Optogenetics

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Channelrhodopsins (ChRs) are microbial type rhodopsins functioning as light-sensitive cation channels in microalgae. Since channelrhodopsins depolarize membranes in the light, they are used as optogenetic tools for generating action potentials in neurons by blue light flashes.
Recently we identified two channelrhodopsin variants in the colonial alga Volvox carteri. One of them named Volvox Channelrhodopsin 1 (VChR1) shows a red shifted action spectra with an absorption maximum at 548 nm. Although there is strong demand for a red-absorbing channelrhodopsin, application of VChR1 has been very limited due to its low expression level in neurons. Now we report about the expression of hybrids comprising fragments of VChR1, VChR2 and Chlamydomonas ChR2 with improved expression level. In addition we identified residues involved in color-tuning. Our goal is to provide ChR variants that in total cover the complete visible spectrum all the way from 400 to 600 nm.

2780-Pos

Bifunctional Properties of Channel rhodopsin 2

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Ever since its first characterization (Nagel *et al.*, 2003), Channelrhodopsin-2 (ChR2) has been used extensively in the light-activated control of neural cells in culture as well as in living animals. Here we describe its dual function as proton pump (in-line with for example Bacteriorhodopsin) and light-gated inward rectifying cation channel. Pump currents could be measured both in electrofused giant HEK293 cells and on planar lipid membranes.
We also present the determination of the wildtype (WT) and mutant single channel conductances under different conditions by means of stationary noise analysis. Whole cell recordings of a HEK293 cell line stably expressing the truncated ChR2 (amino acid residues 1-315), which behaves identically to the full length protein (Nagel *et al.*, 2003), or of semi-stable mutant ChR2 cell lines showed additional noise upon illumination. This noise is related to the opening and closing of the channel. From power spectra, the single channel conductance of was obtained (e.g. 91 ± 25 fS for WT ChR2, -60 mV applied and 200 mM Guanidine$^+$ in the bath solution). The inward rectification could be observed on the scale of the single channel (bath: 200 mM Guanidine$^+$, 0 mV to -60 mV applied). Also, a saturation of the single channel conductance could be observed at high Guanidine$^+$ concentrations.

2781-Pos

P2R in Eosinophils and Possible Role in Migration

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ATP and other nucleotides can be released from cells through regulated pathways or following the loss of plasma membrane integrity. These nucleotides act on P2 family of receptors that are divided in P2X ionotropic receptors and G protein-coupled P2Y receptors. Such receptors have been characterized in many rat immune cells, one exception are eosinophils which are involved in several pathological and physiological processes.
The eosinophils were obtained from peritoneal lavage of wistar rats followed by a purification step of Metrizamide density-gradient centrifugation. Firstly, we have performed an immunofluorescence characterization using antibodies against P2XR and P2YR. The cells were positives for P2X $_{1,2,4 \text{ and } 7}$ and [[Unsupported Character - Codename]]P2Y $_{1,2 \text{ and } 4}$. Our next step was to verify whether those receptors were functional using patch clamp recording which showed that ATP (1504 ± 283 pA/pF)and ATPγS (1231 ± 164 pA/pF) were the most potent agonists where the others elicited little (α,β me ATP, ADP, BzATP, β,γ me ATP, 2me SATP) or no response (UDP, cAMP, adenosine). After that we have tested the participation of these receptors in eosinophils migration *in vitro* (1 or 2h) using a transwell chamber in order to investigate their possible physiological role. ATP and other agonists were able to increase migration, an effect which could be blocked by suramin, a general blocker of P2R. In keeping with this idea, we tested whether they are implicated in the migration of eosinophils using an inflammation model of rat allergic pleurisy. Our results suggest an increase of eosinophils migration induced by ATP. Corroborating with the transwell results, suramin also blocked migration.
As far as we are concerned, this study was the first to demonstrate that rat eosinophils express P2X and P2Y which can increase migration of eosinophils in *vitro* and *in vivo*.

2782-Pos

The First Transmembrane Domain of a Drosophila Innexin is Loosely Packed

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A tryptophan-scanning technique was applied to the first transmembrane domain (M1) ofthe *Drosophila* gap junction protein ShakB(lethal) variant A