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

with the aim of identifying sites of transmembrane domain interaction. The tryptophan-scanning technique is based on the premise that the large bulky side-chain of tryptophan is tolerated when positioned in a lipid environment but disrupts protein function when inserted at a site of protein interaction. Tryptophan was substituted sequentially for sixteen amino acids within M1 of Shak-B(lethal) and channel function was assayed using the *Xenopus* oocyte expression system. Four sites of transmembrane domain interaction were identified, all positioned along the same helical face of M1. The results suggest that M1 interacts closely with only one other transmembrane helix.

2783-Pos

Temperature dependence of Proton Permeation through a Voltage-Gated Proton Channel in Microglia

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Voltage-gated proton channels are found in many different types of cells, where they facilitate proton movement through the membrane. The mechanism of proton permeation through the channel is an issue of long-term interest, but remains an open question. To address this issue, we examined the temperature-dependence of proton permeation. Under whole-cell recordings rapid temperature changes within a few ms were imposed. This method allowed for the measurement of current amplitudes immediately before and after a temperature jump, from which the ratios of these currents (I_{ratio}) were determined. The use of I_{ratio} for evaluating the temperature dependence minimized the contributions of factors other than permeation. Temperature jumps of various degrees (ΔT; −15 - 15°C) were applied over a wide temperature range (4 - 49°C), and the Q_{10}s for the proton currents were evaluated from the I_{ratio}s. Q_{10} exhibited high temperature dependence, varying from 2.2 at 10°C to 1.3 at 40°C, implying that processes with different temperature dependencies underlie the observed Q_{10} (apparent Q_{10}, Q_{10}^{app}). A novel resistivity pulse method revealed that the access resistance with its low temperature dependence became predominated in high temperature ranges. The Q_{10}^{app} was decomposed into Q_{10} of the channel and of the access resistances. Finally, the Q_{10} for proton permeation through the voltage-gated proton channel itself was calculated and found to vary from 2.8 at 5°C to 2.2 at 45°C as expected for an activation enthalpy of 64 kJ/mol. The thermodynamic features for proton permeation through proton-selective channels would provide an important clue for the permeation mechanism.

2784-Pos

Membrane Topology of S4 of the Mouse Voltage-Gated Proton Channel

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VSOP/Hv1 is a voltage-gated proton channel that contains the voltage sensor domain (VSD) but not pore domain. VSD of VSOP/Hv1 allows protons to permeate as well as sensing voltage. It has been reported that basic amino acids in the fourth transmembrane segment (S4) of voltage-gated ion channels play critical roles in voltage-sensing. Mouse VSOP (mVSOP) has three arginine residues (R1, R2, R3) in a pattern similar to those conserved in other voltage-gated channels. To address the role of S4 in mVSOP, we have reported that the truncated construct (A206sop) just downstream of R2 in the S4 is still ion-conductive (Biophysical Society 53th Annual Meeting, 2009). In this study, we further analysed properties of A206stop. The outward current of A206stop was almost completely blocked by zinc. Visualization of intracellular pH using BCECF (pH-sensitive ratiometric dye) showed that cytoplasm of tsA201 cell was alkalinized under the depolarization condition. Na and K ion do not permeate through A206stop. Gating properties of the proton currents through A206stop were sensitive to either intracellular pH or extracellular pH. However, voltage dependency of A206stop was weaker than that of full-length mVSOP, and the I-V relationship of A206stop was shifted rightward. These results indicate that A206stop retains the basic properties of the voltage-gated proton channel even if it lacks a half of S4. We also carried out two biochemical assays: site-directed cysteine-scanning using accessibility of maleimide-reagent as detected by western blotting (pegylation protection) and in vitro glycosylation assays. Both showed that S4 of A206stop inserts into the membrane and the position of A206 faces intracellular aqueous environments. These findings suggest that the region downstream of the R2 position of S4 of VSOP/Hv1 is not essential for proton selectivity.

2785-Pos

Pharmacological Relevant Amantadine Binding Site is in the Pore of Influenza a Virus M2 Proton Channel

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The influenza A virus M2 protein (A/M2) and the influenza B virus BM2 protein are both homotetrameric pH-activated proton channel that facilitates viral uncoating by acidification the interior of endosomally encapsulated virus. Antiviral drugs amantadine and its derivative rimantadine inhibit A/M2 channel of influenza A virus, but not BM2 channel of influenza B virus. The atomic structure of the pore-transmembrane (TM) domain peptide has been determined by X-ray crystallography (Stouffer et al., Nature 451, 596-599 [2008]) and of a larger M2 peptide by NMR methods (Schnell and Chou, Nature 451, 591-595 [2008]). The crystallographic data shows electron density (at 3.5 Å resolution) in the channel pore, consistent with amantadine blocking the pore of the channel. In contrast, the NMR data show four rimantadine molecules bound on the outside of the helices towards the cytoplasmic side of the membrane. Drug binding includes interactions with residues 40-45 and a hydrogen bond between rimantadine and Asp44. These two distinct drug-binding sites led to two incompatible drug inhibition mechanisms. The cytoplasmic binding site predicts that D44 and R45 to alanine mutations would interfere with rimantadine binding and lead to a drug insensitive channel. However, the D44A channel was found to be sensitive to amantadine when measured by TEVC recordings in oocytes of *Xenopus laevis*, and when the D44 and R45 mutations were introduced into the influenza virus genome. Furthermore, two chimeras containing 5 residues of the A/M2 ectodomain and residues 24-36 of the A/M2 TM domain show 85% amantadine/rimantadine sensitivity and specific activity comparable to wt BM2. These functional data suggest the pharmacological relevant amantadine/rimantadine binding site is in the pore of the M2 channel.

2786-Pos

Ryanodine Receptors Control Cytosolic Calcium Elevation Following Activation of Store-Operated Calcium Entry in Activated but not Resting Human T Lymphocytes

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Previously we have shown that in Jurkat T lymphocytes, the ryanodine (RyR) receptors are activated by store-operated Ca^{2+} entry (SOCE) and that inhibition of RyR significantly reduced elevation in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) following SOCE. Because Jurkat T cells differ from normal human T cells, we explored contribution of RyR into Ca^{2+} signaling in two functional human T lymphocyte subsets: resting and activated. Resting T cells were isolated from the peripheral blood of healthy humans and activated in vitro using anti-CD3 and anti-CD28 antibodies. Assessing the $[Ca^{2+}]_i$ dynamics in activated T cells using fura-2, a Ca^{2+} indicator, revealed that RyR blockers ryanodine (Ry) and dantrolene (Da) significantly reduced Ca^{2+} elevation upon SOCE activation, while increasing Ca^{2+} content within the store, which is consistent with our previous findings in Jurkat T cells. In contrast, in resting T cells neither Ry nor Da affected $[Ca^{2+}]_i$ elevation upon SOCE activation at physiological concentration (2 mM) of extracellular Ca^{2+}. However, the inhibitory effects of RyR blockers were observed in resting T cells in the presence of the elevated extracellular Ca^{2+}concentration (10 mM). Using Mn^{2+} quench of fura 2 fluorescence approach we further explored whether inhibition of $[Ca^{2+}]_i$ elevation in the presence of RyR blockers could be attributed to termination of SOCE due to the Ca^{2+} accumulation within the store. We found that rates of Mn^{2+} quench were identical in the presence and absence of RyR blockers, indicating that within a given timeframe enhanced Ca^{2+} accumulation within the store did not affect SOCE. We conclude that in activated human T cells Ry-sensitive store serves as an intermediate compartment for SOCE and that RyR controls $[Ca^{2+}]_i$ dynamics by regulating $[Ca^{2+}]_i$ release from the store.

2787-Pos

Incorporation of RyR2 and Other Ion Channels into Nanopore Based Planar Lipid Bilayers for Low Noise Single Channel Recordings

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Measurement of ion channel activity at the single molecule level in isolated planar lipid bilayers (PLB) is a critical biophysical tool for understanding the function of such proteins. However, current PLB techniques involving bilayers painted across apertures >100 μm suffer from high noise arising from the capacitance of the bilayer and is severely limited in bandwidth. Therefore, it is