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

Miyuki Kuno[1], Hiroyuki Ando[2], Hirokazu Morihata[1], Hiromu Sakai[1], Hiroyuki Mori[1], Shigetoshi Oiki[2].
[1]Osaka City University Graduate School of Medicine, Dept. of Physiology, Osaka, Japan, [2]University of Fukui, Med.Sci., Dept. of Molecular Physiology and Biophysics, Fukui, Japan.

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

Souhei Sakata[1], Tatsuki Kurokawa[1], Morten Nørholm[2], Masahiro Takagi[3], Yoshifumi Okochi[1], Gunnar von Heijne[2], Yasushi Okamura[1].
[1]Dep. Integrative Physiology, Graduate School of Medicine, Osaka University, Suita, Japan, [2]Dep. Biochemistry and Biophysics, Stockholm University, Stockholm, Sweden, [3]National Institute for Physiological Science, National Institute of Natural Sciences, Okazaki, Japan.

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

Chunlong Ma[1], Yuki Ohigashi[1], Xianghong Jing[1], Robert A. Lamb[1,2], Lawrence H. Pinto[1].
[1]Northwestern University, Evanston, IL, USA, [2]Howard Hughes Medical Institute, Evanston, IL, USA.

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

Pratima Thakur, Sepehr Dadsetan, Alla F. Fomina.
Univ. California, Davis, Davis, CA, USA.

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

Prithwish Pal[1], Geoffrey A. Barrall[1], Ariel L. Escobar[2], Melissa A. Poquette[1], Patricio Velez[3], Andrew D. Hibbs[1].
[1]Electronic Bio Sciences, San Diego, CA, USA, [2]University of California at Merced, Merced, CA, USA, [3]Florida State University, Tallahassee, FL, USA.

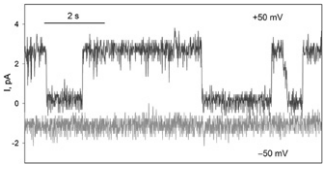
Measurement of ion channel activity at the single molecule level in isolated planar lipid bilayers (PLB) is a critical biophysical tool for understanding the function of such proteins. However, current PLB techniques involving bilayers painted across apertures >100 μm suffer from high noise arising from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

difficult to investigate the fast gating properties of certain channels e.g the Ryanodine Receptor (RyR2). RyR2 channels, which play a key role in the intracellular Ca2+ induced calcium release mechanism, demonstrate a complex gating characterized by bursts of very fast open-close transitions that cannot be resolved by conventional PLB apparatus. Furthermore, the low current amplitude these channels produce in presence of the physiological ion Ca2+ , complicates the analysis.
We have developed a robust platform based on glass or quartz nanopore membranes (GNMs, 200-3000 nm radius pore size), for performing high bandwidth, low noise measurements of such ion channels in lipid bilayers. Previously, we have demonstrated incorporation of bacterial toxins [1] and porins in these small bilayers. Here we report the successful incorporation and measurement of RyR2 activity in such a system. Vesicles prepared from sarcoplasmic reticulum enriched in RyR2 channels were fused through osmotic swelling to PLBs formed on a GNM with a 3000 nm radius orifice, allowing unprecedented resolution of single RyR2 channel events at 10 kHz.
In addition, we have fused vesicles containing nystatin and ergosterol to small PLBs (< 1000nm radius) successfully. The latter method has the potential to provide a general technique for incorporation of a variety of ion channels in small GNM bilayers.
[1] White et al J. Am. Chem. Soc., 129, 11766-11775, 2007

2788-Pos
The Intracellular Loop of Orai1 Plays a Central Role in Fast Inactivation of CRAC Channels
Sonal Srikanth, Hea-Jin Jung, Bernard Ribalet, Yousang Gwack.
UCLA, Los Angeles, CA, USA.
Store-operated Ca^{2+} entry (SOCE) due to activation of Ca^{2+}-release-activated Ca^{2+} (CRAC) channels leads to sustained elevation of cytoplasmic Ca^{2+} and activation of lymphocytes. CRAC channels consisting of four pore-forming Orai1 subunits are activated by STIM1, an endoplasmic reticulum Ca^{2+} sensor that senses intracellular store-depletion and migrates to plasma membrane proximal regions to mediate SOCE. One of the fundamental properties of CRAC channels is their Ca^{2+}-dependent fast inactivation (CDI). To identify the domains of Orai1 involved in CDI, we have mutated residues in the Orai1 intracellular loop linking transmembrane (TM) segment II to III. Mutation of four residues $V^{151}SNV^{154}$ (MutA) at the center of the loop abrogated fast inactivation leading to increased SOCE as well as higher CRAC currents. Point mutation analysis identified five key amino acids $N^{153}VHNL^{157}$ that increased SOCE in Orai1 null murine embryonic fibroblasts. Expression or direct application of a peptide comprising of the entire intracellular loop or the sequence $N^{153}VHNL^{157}$ blocked CRAC currents from both WT and MutA Orai1. A peptide incorporating the MutA mutations had no blocking effect. Concatenated Orai1 constructs with four MutA monomers exhibited high CRAC currents lacking fast inactivation. Reintroduction of a single WT monomer (MutA-MutA-MutA-WT) was sufficient to fully restore fast inactivation, suggesting that only a single intracellular loop can block the channel. These data suggest that the intracellular loop of Orai1 acts as an inactivation particle, which is stabilized in the ion permeation pathway by the $N^{153}VHNL^{157}$ residues. These results along with recent reports support a model in which the N terminus and the selectivity filter of Orai1 as well as STIM1 act in concert to regulate the movement of the intracellular loop and evoke fast inactivation.

2789-Pos
A High Throughput Microfluidic Approach Enables Fast Exchange of Solutions and Ligand Gated Ion Channel Recording from Cell Ensembles
Juliette Johnson, Nianzhen Li, Qin Chen, Ian Spencer,
Cristian Ionescu-Zanetti, Jeff Jensen.
Fluxion Biosciences, South San Francisco, CA, USA.
Electrophysiology is the preferred technique for characterizing ion channel function and kinetics. It is the most functionally pertinent assay for screening in terms of information content. High throughput pharmaceutical screens often use a population patch approach, which eliminates cell-to cell variability of single cell recordings. However, currently available population patch platforms have key shortcomings such as a) the inability for fast exchange of solutions, b) the inability to apply multiple compounds to the same ensemble of cells, and c) the inability to record fast desensitizing channels.
Here we present novel data showing that by using a microfluidic network design along with population patch recording we are able to overcome these obstacles. We validated our system using cells expressing voltage-gated channels in ensembles of up to 30 cells under voltage clamp. Moreover, these results showed that there is fast compound application (<100ms). The time course of compound application was confirmed using fluorescent indicators and biological reporters, such as GABA-A expressing cells. These data also validated our ability to record from fast desensitizing ligand gated ion channels without appreciable desensitization. We compared the time course of solution exchange with and without a protective layer technique and additionally characterized application of multiple compounds to the same ensemble of cells. In conclusion, the novel microfluidic approach allows for the fast exchange of compounds and facilitates the recording of fast activating voltage and ligand-gated channels.

2790-Pos
Components of *E. Coli* Energy-Transducing Complexes, ExbB and TolQ, Display Ion Channels
Stanislav D. Zakharov[1], Herve Celia[2], Kirill A. Datsenko[1],
Monica Santamaria[3], Julian Whitelegge[4], Roland Lloubes[2],
William A. Cramer[1].
[1]Purdue University, West Lafayette, IN, USA, [2]Inst Biol Struct Microbiol, CNRS Aix-Marseille Univ, Marseille, France, [3]CIMA, Univ Navarra, Pamplona, Spain, [4]David Geffen School of Medicine, UCLA, Los Angeles, CA, USA.
ExbBD/TonB and TolQR/TolA complexes of the cytoplasmic membrane transduce energy stored in the electrochemical proton gradient to drive cellular import of siderophores or maintain outer membrane barrier function. Both complexes are utilized for cellular import of colicins. It is unknown how they exert energy transducing and import functions.
We explore the idea that energy-transduction by ExbBD/TonB and TolQRA is coupled to ion- translocation through ion channels formed by transmembrane helices of ExbB and TolQ. Plasmid-expressed ExbB and TolQ were extracted with detergent from membranes, purified, and reconstituted into liposomes. For channel measurements proteoliposomes were fused to planar lipid membranes. Changes in protein tertiary structure upon membrane reconstitution were detected by thermal melting of alpha-helices using far-UV circular dichroism. ExbB reconstituted into liposomes, in contrast to its behavior in detergent, melted cooperatively, implying inter-helix interactions. ExbB and TolQ displayed cation-selective ion channels of small conductance (Figure). Divalent cations decreased channel conductance.
Channel formation was more prominent at pH<6. It is proposed that transmembrane ion current through ExbB and TolQ channels is transduced into conformational changes of periplasmic domains of the membrane-anchored TonB and TolA components of the complexes.



2791-Pos
Phases, Transport, and Dielectric Properties of Water Confined in Nanoscale Channels
Robert J. Mashl, Eric Jakobsson.
U. Illinois, Urbana, Urbana, IL, USA.
Biological channels formed from membrane proteins assemble into complex conduits for passage of select ions and molecules across the cell membrane. The selectivity properties of some of these channels has inspired the search for synthetic analogs that may serve as nanoscopic filters for various technical applications, including electronic devices and desalination membranes. To understand better the properties of water confined to nano-sized channels, we study the structure and dynamics of water inside long, hydrophobic channels under ambient pressure and temperature using classical molecular dynamics simulations. We find that water undergoes distinct transitions in structure and dynamic properties as the channel diameter is varied and describe the resulting anisotropic properties of the water in these confined geometries.

2792-Pos
Computational Evaluation of Nanopore Conductivity in Electroporation
Malgorzata Kotulska, Witold Dyrka.
Wroclaw University of Technology, Wroclaw, Poland.
Intracellular transport of molecules hardly permeant through plasma membrane could be significantly enhanced by application of electric field, inducing membrane electroporation (EP). Electroporation has been applied to amplify the insertion of nucleic acid molecules in genetic modifications, drug transport in cancer treatment, and immune stimulation. Optimal protocol of EP should be selected with regard to the application, taking into account membrane composition and physico-chemical properties of transported molecules. Various probes have been applied to test the permeability and selectivity of the molecular transport through electropores. They are used for selecting optimal protocol and obtaining the electropore characteristics, such as the pore median radius and their density. To accomplish this task with high accuracy, an appropriate theoretical model should be implemented. In case of electropores there are two main difficulties to be addressed. First