more direct role of $G_{i/o}$ proteins in TRPC4/C5 activation is supported by the demonstration that $G\alpha_i$ and $G\alpha_o$ subunits physically bind to the C-terminus of TRPC4. We suggest that a concerted action of $G_{q/11}$- and $G_{i/o}$-mediated signaling pathways is required for the full activation of TRPC4/C5, making these channels coincident detectors of multiple environmental cues. We have tested the co-dependence of native TRPC4/C5-like currents in smooth muscle cells, endothelial cells and neurons. (Supported by AHA Grant-in-Aid 0755277B and NIH grant RO1 DK081654)

1697-Pos
Function of Ion Channels in Cell Migration
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The prognosis of tumor disease is greatly influenced by the formation of metastases. A critical step of the socalled metastatic cascade is the ability of tumor cells to migrate away from the primary tumor. Ion channels and transporters are an an integral part of the cellular migration machinery that complement and regulate other components of the cellular motor. KCa3.1 channels for example are upregulated in many tumor cells. Their inhibition slows down migration and prevents its chemokinetic acceleration. KCa3.1 channel activity supports migration among others by inducing localized changes of cell volume at the rear part of crawling cells. TRPC1 channels are involved in coordinating the movement of the protruding front withe retracting rear part of migrating cells. Thus, genetic ablation of channels leads to a marked impairment of persistent migration. In addition, TRPC1 channels are also part of the cellular compass during directed migration in a chemotactic gradient. Silencing of TRPC1 activity by means of RNA interference or pharmacologically with GsMTx-4 impairs chemotaxis towards FGF-2. Reducing TRPC1 channel activity blocks directed cell migration as efficiently as does inhibition of particular steps of growth factor-induced signaling like phospholipase C or phosphatidylinositol-3-OH kinase. Taken together, ion channels are crucial for multiple aspects of tumor cell motility.

1698-Pos
A Combined Spectroscopic and Biochemical Approach to Counting MscL Subunits
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The mechanosensitive channel of large conductance (MscL) is a homooligomeric, stretch activated membrane protein responsible for regulating osmotic pressure in bacteria and archaea. Increasing membrane tension activates the protein resulting in a ~2.9 nS non-selective pore. Two MscL crystal structures have been solved in distinct conformations and oligomeric states. M. tuberculosis MscL is a non-conducting pentamer while S. aureus MscL is a partially expanded tetramer. The primary sequences of these proteins are 38% identical and 57% similar. Given their high relatedness, the structures raise interesting questions regarding the assembly and activation of MscL homologs. We have been interested in understanding the molecular determinants responsible for the differences between the two structures, specifically the switch between tetrameric and pentameric species. Using a combination of multi-angle light scattering and mass tagging followed by Blue Native PAGE, we have characterized the oligomeric states of several MscL homologs and chimeras. We find that the two methods are in good agreement with each other and single molecule measurements. Potential reasons for the differences in oligomeric states will be discussed.

1699-Pos
TREK Channel Pore Probed by Cysteine Scanning Mutagenesis and Structural Modelling
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The TREK channel belongs to the superfamily of two-pore-domain potassium channels (K2P-channels) that are made up of four transmembrane segments (TM1 - TM4) and two pore-forming domains that are arranged in tandem. The activity of these channels is directly regulated by the intracellular pH, heat, polyunsaturated fatty acids, phospholipids and mechanical stretch. Currently little is known about the pore structure and how these different stimuli gate the pore in structural terms. To this end we employed systematic cysteine scanning mutagenesis on the four TM domains and functionally characterised these mutants in several respects: I) using chemical cysteine modification we identified pore lining residues, II) by measuring detailed pH dose response curve we identified residues involved in the pH gating mechanism and III) by studying different pore blocking compounds we identified potential blocker interacting residues. These sets of functional data will be evaluated in the context of structural models of the TREK channel pore in the closed and open state.

1700-Pos
Coupling of Water Permeation with Mechano-Gating In the E-Coli Mechanosensitive Channel MscL
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The bacterial mechanosensitive channel of large conductance MscL is constituted of homopentamer of a subunit with transmembrane inner and outer α-helices, and its 3D structure of the closed state has been resolved. Understanding the gating process driven by tension in the membrane is one of the major subjects in MscL study. Although several models for its opening process have been proposed based on molecular dynamics (MD) simulations, as they do not include MscL-lipid interactions, it remains unclear which amino acids sense membrane tension and how the sensed force induces channel opening. We performed MD simulations for the mechano-gating of MscL embedded in the lipid bilayer. Upon tension generation in the bilayer, Phe78 in the outer helix was dragged by lipids, leading to a tilting of the helices. Among amino acids in the outer helix, Phe78 at the water-lipid interface showed the strongest interaction with lipids, thus may work as a major tension sensor. Neighboring inner helices cross each other in the inner leaflet, forming the most constricted part of the pore. In the closed state of MscL, Leu19 and Val23 in the constricted part form stable hydrophobic environment. As tension increased, the crossings moved toward the cytoplasm associated with an expansion of the constricted part and the hydrophobic environment was broken followed by water penetration and permeation. It seemed that water penetration and permeation accelerated the pore opening probably by decreasing the hydrophobic interaction between Leu19 and Val23. We performed MD simulations of the GOF mutant G22N with almost the same pore size of the wild type, and found that G22N mutant permeated water molecules without tension increase in the bilayer. This spontaneous water permeation seemed to be mediated by hydrogen bonds between Asn22 and water molecules.

1701-Pos
Significance of the *Corynebacterium Glutamicum* YggB Protein in Fine-Tuning of Compatible Solute Accumulation
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Based on structural similarity, the *yggB* gene product of *Corynebacterium glutamicum* belongs to the family of MscS-type mechanosensitive channels. In order to clarify its physiological significance in response to osmotic shifts in detail, we studied the properties of YggB using both patch-clamp techniques and betaine efflux kinetics. After heterologous expression in an *E. coli* strain devoid of mechanosensitive channels, in patch-clamp analysis of giant *E. coli* spheroplasts YggB showed the typical pressure dependent gating behavior of a stretch-activated channel with a current/voltage dependence indicating a strongly rectifying behavior. Apart from that, YggB is characterized by significant functional differences with respect to conductance, ion selectivity and desensitation behavior as compared to MscS from *E. coli*. Deletion and complementation studies in *C. glutamicum* showed a significant contribution of the YggB protein to betaine efflux in response to hypoosmotic conditions. As a novel finding, detailed analysis of concomitant betaine uptake (by the betaine transporter BetP) and efflux (by YggB) under hyperosmotic conditions revealed that YggB acts as a key player in osmoregulation in *C. glutamicum* by fine-tuning the steady state concentration of compatible solutes in the cytoplasm which are accumulated in response to hyperosmotic stress.