mutants is ongoing but has already demonstrated dramatic effects on TREK channel gating and its regulation by a range of known modulators. The results also point towards a dominant role for the inactivation gate (i.e. selectivity filter) in TREK-1 channel gating.

1523-Pos Board B433
Effect of Intracellular pH on the Mechano Activated Potassium Channel from Human Erythrocyte
Jesus G. Romero, Angeles R. Zambrano.
We have presented direct evidences of the existence of a Mechano-Activated K+ Channel in Human Erythrocytes (HEMKCA)(1). This channel presents a sigmoid dependence of Po on applied pressure and a conductance of 17pS in symmetrical 120mM KCl, 12mM NaCl, 1.8mM CaCl2, 10mM Tris, pH 7.3. We have proposed that this channel plays a fundamental roll in the senescent process of this cell, as the molecular mechanism behind to its biological clock (The K+ hypothesis). Because of the interplay of O2 and CO2 at microcirculation level it is expected a change in free H+ activity, and it is been already presented a difference in internal pH (pHi) between younger (pHi=7) and older cells (pHi=7.3)(2). Here, using the Patch-Clamp technique we have studied the effect of intracellular pH, ranging from 7.3 to 6.5, on the activity of HEMKCA. This decrease of pHi result in a dramatic decrease of Po, been equal to 0 at pHi lowers than 6.5. The open probability presents a sigmoid dependence on pH and can be described as a titration curve in concordance with the protonation of one or more independent sites, presenting a pK of 6.6, this suggests the involvement of the side-chain imidazole group of Histidine. The fact that there was no change in the channel conductance suggests that the titrated site(s) is not close to the mouth, the vestibule or the pore of the channel. In conclusion, we present a dramatic dependence of Po with pHi, so in younger cells the channel activity is naturally depressed compared with older cells in concordance with the K+ hypothesis presented by us.
(1) Romero, J.G. and Romero, P.J.(2005)Biophysical J. 88(1): 266a
(2) Romero, P.J. and Romero E. (2004) Act Cient Venez. 55(1):83,5

1524-Pos Board B434
Understanding the Mechanotransductional Basis of Intravascular Bubble Injury
Alexandra L. Klinger, Benjamin Pichette, David M. Eckmann.
Intravascular bubbles, commonly introduced during surgery or precipitated from dissolved gases in decompression sickness, can occlude vessels, diminish perfusion, and initiate thrombotic and inflammatory pathways. The specific interactions between bubbles and the endothelium that lead to cell injury and death are poorly understood. We report live single cell time-lapse imaging of human umbilical vein endothelial cells (HUVECs) perturbed by microbubbles produced by injecting air through a pulled glass capillary ground so that its bevel opposes the buoyant force of the bubble. The bubble is moved with a three-stage micromanipulator into contact with a cell containing Fluo-4, a calcium sensitive dye, and imaged using phase-contrast and fluorescence microscopy. A significant transient elevation in intracellular calcium is observed in response to bubble impact. Membrane depolarization (external K+, 130 mM) does not block the calcium response, indicating that voltage-dependent calcium channels are not involved. Extracellular calcium and an intact actin cytoskeleton are required for the elevation of intracellular calcium upon bubble impact, which is reduced with the addition of gadolinium, a general inhibitor of mechanosensitive channels. These results suggest that a combination of the mechanical deformation and the air-water interface of the bubble activate a plasmalemma ion channel triggering further release of internal calcium stores. The calcium response is ameliorated with addition of surfactant, likely competing for occupancy of the air-liquid bubble interface. Supported by ONR N00014-08-1-0436 and NIH R01 HL67986.

1525-Pos Board B435
The Mechanosensitive Channel Piezo1 and the Effect of Gsmtx4
Philip A. Gottlieb, Chilman Bae, Frederick Sachs.
Piezo1 is a mechanically activated nonselective cation channel isolated from NEURO2A cell line (1). We measured the kinetic response to mechanical stimuli using a high speed pressure clamp. Expressing a clone of Piezo1 (courtesy Armand Patapoutian) in BAEC cells we compared them the responses to endogenous channels in the NEURO2A cells. The transfected BAEC cells responded to mechanical stress in cell-attached mode consistent with expression of Piezo1, having similar pressure sensitivity and voltage dependent inactivation. We modeled channel activity using a linear 3 state model of closed, open and inactivated with the closed to open state transition as pressure sensitive. Outside-out patches from transfected cells behaved similarly suggesting that the details of cytoskeletal structure are not critical to channel function. With outside-outs the pressure activation curve right shifted to a increased pressure as expected since the radius of curvature of outside-outs is smaller than inside-outs. We also showed that GsMTx4, an inhibitor of mechanical channels, inhibited Piezo1 currents in Neuro2A transfected cells. At a 2.5 μM we observed 75% inhibition of peak channel activity, and the effect was reversible with wash out rates on the order of seconds.
1. Coste et al. Piezo1 and Piezo2 Are Essential Components of Distinct Mechanically Activated Cation Channels (2010) Sci 330 55-60.

1526-Pos Board B436
TRPV1Δ1-283: A Candidate Osmoreceptor Channel
Cristian A. Zaelzer, Pierce Hua, Maria Prager-Khoutorsky, Sorana Ciura, Sukhee Lee, Wolfgang Liedtke, Charles W. Bourque.
Mammalian osmosensory neurons exposed to hypertonicity display a shrinking-induced increase in non-selective cation conductance. That causes membrane depolarization and excitation triggering homeostatic responses such as thirst and antidiuretic hormone release. The molecular identity of the osmoreceptor channel is not known, but appears to involve a capsaicin-insensitive N-terminal variant of the transient receptor potential vanilloid type 1 (TRPV1) channel (Ciura and Bourque, 2006; Sharif-Naeini et al., 2006). Here, we report that heterologous expression of TRPV1 variant Δ1-283, which lacks exons 1-5, confers osmosensory characteristics similar to those found in native osmosensory neurons. Whole cell voltage clamp recordings were used to examine the effects of hyperosmolality (+mannitol) on human embryonic kidney (HEK293) cells expressing Δ1-283 TRPV1, GFP or wild type TRPV1. Dynamic imaging confirmed that all HEK cells exposed to hypertonicity underwent visible shrinking. However in contrast to GFP (n = 10) and TRPV1 (n = 32) transfected cells, which were unresponsive, ~41% (84/206) of cells transfected with Δ1-283 TRPV1 showed a progressive and sustained increase in non-selective cation current and membrane conductance when exposed to a hyperosmotic stimuli lasting 2-5 minutes. The effects of osmolality were dose-dependent (+5 to +80 mosmol/kg), mimicked by suction induced cell shrinking (n=6), and were abolished by Ruthenium Red. Moreover, addition of capsaicin caused robust responses in cells expressing TRPV1, but had no effect on those expressing Δ1-283 TRPV1. Imaging experiments on cells loaded with Fura-2 revealed that ~21% of the cells transfected with Δ1-283 TRPV1 display reversible increases in intracellular [Ca^{2+}], whereas ~17% shown an increase without reversion when exposed to a +40 mosmol/kg hyperosmotic stimulus. These results suggest Δ1-283 TRPV1 channels could mediate mechanically-induced increases in cation current associated with hypertonicity-induced shrinking in osmosensory neurons.

1527-Pos Board B437
Analysis of Novel Mechanosensitive Channel Activities in Escherichia Coli
Michelle D. Edwards, Ian R. Booth.
Bacterial mechanosensitive channels gate in response to rapid increases in membrane tension that occur when cells transfer from high to low osmolarity environments. They open large, non-specific pores in the cytoplasmic membrane, releasing small solutes and ions to halt the instantaneous water influx that would otherwise cause cell lysis. Two major mechanosensitive channel families exist in bacteria (MscL and MscS) and protein databases show that *Escherichia coli* possess one MscL and six MscS family members. MscL was identified in *E. coli* in 1994 and our group has since cloned three of the MscS homologues: MscS, MscK and YbdG. These channels have been extensively studied and patch-clamp analysis on membranes of giant protoplasts shows that each gates at different levels of pressure and is associated with a specific conductance. MscL and MscS activities appear frequently in recordings and MscK is also regularly seen. Activities associated with YbdG protein expression are more elusive. However, during electrophysiological recordings additional openings are occasionally observed. Due to their rare occurrence, little is known about these extra pressure-induced currents or their genetic origins. Here we use *E. coli* deletion strains and a modified protoplast preparation protocol to investigate previously uncharacterised mechanosensitive activities in *E. coli* using patch-clamp. When *mscL mscS mscK ybdG* null mutants are tested, infrequent mechanosensitive openings detected can be placed into three categories based on current amplitude and open-dwell properties. Incubation of cells in 0.5M NaCl during

protoplast formation increases the probability of mechanosensitive activity in a patch. Furthermore, a new mechanosensitive channel conductance (~1nS) is revealed in ~40% of patches. Tests so far indicate that 0.5M KCl incubations do not cause the same effect. Further analysis of these novel activities will enhance our knowledge of mechanosensitive channels in bacteria.

1528-Pos Board B438

Combining Scanning Probe and Confocal Microscopy to Investigate the Biophysical Properties of the Primary Cilium

Stephen J. Kocher, David N. Sheppard, Terry J. McMaster.

Primary cilia are non-motile specialised sensory organelles that protrude from the surface of epithelial cells. In the kidney, they are 200 nm in diameter and extend approximately 10 μm in length into the lumen of the nephron. Ciliary dysfunction is linked to autosomal dominant polycystic kidney disease, the most common of the inherited cystic diseases. The manner in which the cilium bends in response to fluid flow along the nephron and the mechanisms leading to the intracellular calcium release are widely debated. To gain insight into the mechanism of ciliary bending, we investigated the biophysical properties of the primary cilium. Here, a TCS SP5 Confocal Microscope (Leica, Wetzlar, Germany) was used to identify cells with a primary cilium and then position a cantilever in a Nanowizard II Atomic Force Microscope (JPK, Berlin, Germany) in the near vicinity of the cilium. We controllably probed individual primary cilia with the AFM cantilever tip to obtain a vertical deflection map in one plane. By probing primary cilia at different heights from the surface of the cell, we produced AFM deflection volume maps. This detailed probing of the mechanical response of the cilium is a mechanical analogue of the z-stack optical imaging employed in confocal microscopy. Thus, using our technique, we have quantified the rigidity of the primary cilium.

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1529-Pos Board B439

Bacteria Regulate Turgor Pressure in Order to Grow

Teuta Pilizota, Joshua W. Shaevitz.

The extreme concentrations of chemicals in a bacterium's cytoplasm generate an osmotic pressure that inflates the cell. It is thought that *E. coli* use a number of interconnected systems to adapt to changes in external pressure, allowing them to maintain turgor and live in environments that range more than two-hundred-fold in external osmolality. To date, how this adaptation is achieved and why pressure is required for survival have been poorly addressed. Previous measurements of osmoregulation in bacteria have been unable to directly probe a single cell's adaptation, focusing instead on the activity of various transporters, or changes in population growth rates. I will show that different mechanisms used by bacteria can be explored using fluorescence imaging to monitor changes in cell shape during adaptation on a single cell level with a time resolution on the order of seconds. This type of measurement allows the different adaptation pathways to be studied individually as well as in defined groups. Furthermore, I will demonstrate that using a number of interconnected systems; bacteria actively regulate their turgor pressure to a preset value, despite changes in their local environment. This precise value of the turgor pressure is required for cell growth and I will make direct connections between pressure adaptation and growth rate in challenging environments.

1530-Pos Board B440

Molecular Organization of the Distal Cilium, a Potential Mechanotransduction Site, in Campaniform Receptor of Drosophila Melanogaster

Xin Liang, Johnson Madrid, Henri Saleh, Jonathon Howard.

The observation of a very short latency between stimulus and cell response in mechanosensation led to the gating-spring concept. The central idea is that the gating of mechanosensitive channels is directly controlled by a compliant structure, the gating spring. This model suggests a transduction apparatus based on the physical organization of several molecular components. We are studying the identities and organization of these molecular components by using campaniform receptor in fly as a model system.

The distal cilium of campaniform receptor is in close proximity to the cuticle and directly connects to the extracellular matrix. We found that NOMPC, a candidate of the mechanotransduction channel, exclusively localizes to this region by immunofluorescence with an antibody against N-terminal of NOMPC. We also found that the electrical response of campaniform receptors is absent in *nompC* mutant. These observations suggest that the distal cilium is the transduction site in the campaniform receptor. Transmission EM of this region reveals a particular microtubule-based structure and membrane-microtubule connection (MMC), which we have analyzed quantitatively using image analysis software. This method was applied to several mutants. First, ultrastructural defects in both microtubule-based structure and MMC in this region were found in a *nompC* mutant, suggesting an important role of NOMPC in the structural organization of this region. Second, the microtubule-based structure in DCX-EMAP mutant is disrupted. Although the localization of NOMPC seems normal, the electrical response of campaniform receptor in DCX-EMAP mutant is altered in both amplitude and time course, suggesting that microtubule structure is involved in shaping the electrical response. In summary, our data provide further information about the general architecture of the transduction site in campaniform receptor and the possible roles of several molecular components in its organization.

1531-Pos Board B441

Role of Muscle Specific Caveolin-3 in Mechanosensitive Channel Regulation

Thomas M. Suchyna, HaiXia Huang.

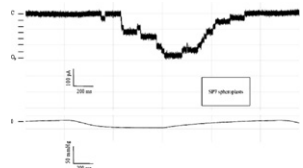
Caveolin-3 (Cav-3) is a small muscle specific scaffolding protein that acts to complex signaling proteins within cholesterol rich raft domains through a small cytoplasmic binding domain. Cav-3 has the unique function of inserting only into the inner membrane leaflet causing expansion of the inner surface that induces curvature and membrane involutions called caveolae. In muscle the dystroglycan complex is localized to the caveolae and is the cortical cytoskeleton attachment point for dystrophin and other proteins which regulate sarcolemma tension. These attributes make caveolae ideal mechanosensing structures and likely locations for mechanosensitive channels (MSCs). To understand the role of Cav-3 in MSC regualtion we depleted membrane cholesterol with methyl β-cyclodextran which caused disruption and internalization of the caveolae protein and increased MSC activity in patch pipette recordings. Overexpression of a Cav-3-GFP fusion protein in well differentiated myotubes also leads to increased MSC activity. Reduction of Cav-3 with miRNA produced no change in MSC activity, though baseline MSC activity is already low in differentiated myotubes. To rapidly and specifically inhibit Cav-3 interactions, we fused the 20 amino acid Cav-3 scaffolding domain the 16 amino acid antennapedia membrane translocation signaling domain (Anten-CSD). Treatment of myotubes with 20 μM Anten-CSD caused internal Ca^{2+} to increase, large blebs to formed on the sarcolemma and Cav-3-GFP aggregation on the surface. There was an increase in MSC activity within 20 minutes after Anten-CBD treatment. We are currently investigating a link between Cav-3 and TRP channels. These studies suggest that Cav-3 may be involved in both localization of MSCs to the sarcolemma and regulating channel activity in myotubes through its ability to complex MSCs with the cortical cytoskeleton.

1532-Pos Board B442

Patch-Clamp Characterization of the MscS-Like Mechanosensitive Channel from *Silicobacter Pomeroyi*

Evgeny Petrov, Dinesh Palanivelu, Paul R. Rohde, Maryrose Constantine, Dan Minor, Boris Martinac.

Based on structural similarity, the Sp7 gene product of a sulphur-compounds decomposing Gram-negative marine bacterium *Silicibacter pomeroyi* belongs to the family of MscS-type mechanosensitive channels. In order to clarify in detail its physiological significance in response to membrane tension we have studied the properties of the Sp7 channel protein using patch-clamp technique. After heterologous expression in MJF465 *E. coli* strain (triple KO E. coli strain: *mscL*$^-$, *mscK*$^-$ and *mscS*$^-$knock-out) devoid of mechanosensitive channels MscL, MscS and MscK, patch-clamp analysis of giant *E. coli* spheroplasts Sp7 showed the typical pressure dependent gating behaviour of a stretch-activated channel (Figure) with a current/voltage dependence indicating a rectifying behaviour similar to MscS from *E. coli*. Except that, Sp7 is characterized by functional differences with respect to conductance and ion selectivity and desensitisation behaviour as compared to MscS.



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