

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

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We have presented direct evidences of the existence of a Mechano-Activated K<sup>+</sup> 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 CaCl<sub>2</sub>, 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<sup>+</sup> hypothesis). Because of the interplay of O<sub>2</sub> and CO<sub>2</sub> at microcirculation level it is expected a change in free H<sup>+</sup> 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<sup>+</sup> 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

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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<sup>+</sup>, 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 de-

pendent 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<sup>2+</sup>], 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