reported in patients suffering from arrhythmias and heart failure. We previously showed that TNFα reduces the repolarizing K^+ current in mice; however the effects of cytokines on other cardiac ionic currents and the mechanism by which these effects are mediated remain incompletely understood. Thus, our objective was to investigate the role of TNFα and IL-1β on L-type calcium current (ICaL), a key player in cardiomyocyte excitation-contraction coupling.
Methods: Cultured neonatal mouse ventricular myocytes were treated with a pathophysiological concentration (30 pg/mL) of TNFα and IL-1β for 24H. ICaL was recorded using the voltage-clamp technique.
Results: The density of ICaL (pA/pF) was decreased by 36% in IL-1β-treated myocytes compared to controls whereas TNFα had no effect. The CaV1.2 mRNA expression was unchanged by IL-1β treatment however there was a significant increase in intracelluar ROS. The antioxidant N-acetyl-L-cystein reduced ROS and restored ICaL density. Furthermore, Western blot experiments reveal that IL-1β increases PKCε membrane translocation. Treatment with a specific PKCε translocation inhibitor or pertussis toxin, the Gαi inhibitor, also reversed the effects of IL-1β.
Conclusion: IL-1β significantly decreased ICaL density without affecting the expression of Cav1.2. Our results suggest that IL-1β mediates its effects by ROS signalling implicating Gαi and subsequently PKCε activation. These findings could contribute to explain the role of IL-1β in the development of arrhythmia and heart failure.

2801-Pos Board B493
A Novel $Na_V1.1$ Mutation L1613P Associated with Familial Hemiplegic Migraine
Chunxiang Fan, Frank Lehmann-Horn, Karin Jurkat-Rott.
Division of Neurophysiology, University ULM, ULM, Germany.
Familial hemiplegic migraine (FHM) is a rare autosomal dominant migraine subtype with aura associated with reversible hemiparesis. It is caused by missense mutations in the genes of *CACNA1A* (FHM1), *ATP1A2* (FHM2) and *SCN1A* (FHM3). Prevailing proposal of FHM pathogenesis is that such mutations could either increase susceptibility of cortical spreading depression (CSD) or facilitate subcortical propagation of CSD. Moreover, all 3 types of FHM showed associated epilepsy. *SCN1A* is a well-known epilepsy gene with over 100 known mutations, while until now only few FHM3 mutations have been identified. Here we report a novel $Na_v1.1$ L1613P mutation in a three-generation family with four patients who present with both FHM and epilepsy. To explore the underlying mechanism, $Na_v1.1$ and the mutation L1613P together with the auxiliary subunits β1 and β2 were cotransfected in human TSA 201 cells. Whole-cell patch clamp recordings showed non-inactivated depolarization-induced sodium currents for L1613P. The present study provides further evidence that SCN1A mutations can cause both FHM and epilepsy, and we propose the inactivation changes in L1613P might be involved in the pathomechanisms of both FHM and epilepsy.

Other Channels

2802-Pos Board B494
A Lack of Significant Lipid Interactions in the Open State of MSCS Implies a Jack-In-The-Box Type Channel Gating Mechanism
Hannah R. Malcolm[1,2], Paul Blount[1], Joshua A. Maurer[2].
[1]UT Southwestern, Dallas, TX, USA, [2]Washington University in St. Louis, St. Louis, MO, USA.
Bacterial mechanosensitive channels are important for cell survival in changing osmotic environments. For the mechanosensitive channel of small conductance from Escherichia coli (Ec-MscS), seven residues have previously been shown to form important lipid contacts in the closed state of the channel. Based on open state crystal structures and closed state models, these residues interact with lipids in the closed state of the channel. Decreasing the hydrophobicity of these residues reduces bacterial survival upon osmotic downshock. Since the closed state appears to be stabilized by lipid interactions, we hypothesized that similar stabilizing lipid interactions could be identified in the open state. Using a computational model of open state Ec-MscS embedded in a lipid bilayer, eleven residues were determined to be lipid exposed with ten of these residues being unique to the open state of the channel. To identify the role of lipid interactions these residues were mutated to alanine and leucine to alter their interaction with the hydrophobic lipids. The effects of these mutations on channel function were assayed using osmotic downshock lysis assays, growth assays, and patch clamp electrophysiology. Mutations of the ten residues that are exposed to the lipid bilayer only in the open state of the channel effected a wildtype phenotype in all of these assays. The lack of phenotypic changes, for residues that interact with the lipid bilayer solely in the open state, suggests that these interactions are not critical for channel gating. This has led us to propose a "Jack-In-The-Box" model of gating for MscS, in which intrinsic lipid bilayer pressure holds the channel in the closed state. Upon relief of the intrinsic bilayer pressure by application of opposing extrinsic tension, MscS springs into the open state.

2803-Pos Board B495
Following the Global Structural Changes of an Ion Channel During its Gating by using a Novel Mass Spectrometry Approach
Duygu Yilmaz[1], Albert Konijnenberg[2], Helgi Ingólfsson[1], Anna Dimitrova[1], Siewert J. Marrink[1], Frank Sobott[2], Armağan Koçer[1].
[1]University of Groningen, Groningen, Netherlands, [2]University of Antwerp, Antwerp, Belgium.
Mechanosensitive ion channels are pore forming membrane proteins playing vital roles in all forms of life. They sense the mechanical force in the lipid bilayer and translate this force into big structural changes. Revealing these structural changes, thus; how these channels work is of great importance for understanding mechanosensation.
Mechanosensitive channel of large conductance (MscL) is such a channel in bacteria, which opens a temporary pore as large as 3-4 nm in diameter in response to hypoosmotic shock. In order to form such a big opening, the channel undergoes drastic structural rearrangements. The methods currently used to study MscL gating such as patch clamp, disulfide crosslinking, FRET spectroscopy, SDSL-EPR enabled researchers to gain information on the local structural changes taking place during channel gating. However, a method for direct observation of the overall global structural changes is lacking. Here, we developed a novel approach to track the global structural changes taking place when MscL goes from the closed to the open state. Our method is based on determining the mass and rotationally averaged size of the ion channel in its intact form using non-denaturing electrospray ionization coupled with ion mobility mass spectrometry (IM-MS). We studied native MscL in its closed form and heteropentameric MscLs in different open states. We could detect for the first time i) the native mass, hence the oligomeric state, of MscL; ii) the global structural changes during MscL gating; and iii) functioning of MscL in the absence of a lipid bilayer. We believe our novel approach opens new avenues for further studies on the dynamic structures of many other membrane proteins, which were so far unattainable by other methods.

2804-Pos Board B496
The Rate of Osmotic Shock Determines Bacterial Survival
Heun Jin Lee, Maja Bialecka-Fornal, Rob Phillips.
California Inst. Tech, Pasadena, CA, USA.
Mechanosensitive (MS) channels allow cells to sense and respond to environmental changes. In bacteria, these channels are believed to protect against an osmotic shock. The physiological function of these channels has been primarily characterized by a standardized assay, where aliquots of batch cultured cells are rapidly pipetted into a hypotonic medium. Under this method, it has been inferred many types of MS channels (MscS homologs in *E. coli*) demonstrate questionable effectiveness against shock. We introduce a single-cell based assay which allows us to control how fast the osmolarity changes, over time scales ranging from a fraction of second to several minutes. We find that the protection provided by MS channels depends strongly on the rate of osmotic change, revealing that, under a slow enough osmotic drop, even "ineffective" MscS homologs can lead to survival rates comparable to those found in wild-type strains. Further, after the osmotic downshift, we observe multiple death phenotypes, which are inconsistent with the prevailing paradigm of how cells lyse. Both of these findings require a re-evaluation of our basic understanding of the physiology of MS channels.

2805-Pos Board B497
Gating Mechanism of Mechanosensitive Ion Channels Studied by Continuum Mechanics
Navid Bavi[1], Takeshi Nomura[2], Qinghua Qin[3], **Boris Martinac**[1].
[1]Molecular Cardiology and Biophysics Division, Victor Chang Cardiac Research Institute, Darlinghurst, NSW, Australia, [2]Department of Molecular Cell Physiology and Bio-Ionomics, Kyoto Prefectural University of Medicine, Kyoto, Japan, [3]College of Engineering and Computer Science, Australian National University, Canberra, Australia.
To complement the existing experimental and computational methods used for studies of membrane protein structure and dynamics a finite element (FE) model for multi length-scale and time-scale investigations of the gating mechanism of mechanosensitive (MS) ion channels has been established. The main advantage of the FE simulation over molecular dynamic simulation