inhibited by 60-120 microM phloretin, and is decreased by ClC-3 expression. Thus, I_{acid} is readily distinguished from expressed ClC-3 transport. These data indicate that ClC-3 H^+ coupling and transport is influenced by anion interaction, and by external protons, as suggested for the bacterial ClCec-1.

2217-Pos Board B354

Energetics and Mechanism of Permeation across FNT Channels

Kalina Atkovska, Jochen Hub.
Institute for Microbiology and Genetics, Georg-August-University of Goettingen, Goettingen, Germany.

The formate-nitrite transporters (FNTs) represent a widespread family of membrane proteins involved in the translocation of monovalent polyatomic anions, such as formate, nitrite and hydrosulfide. Recently solved structures of five of its members reveal a pentameric protein organization with remarkable structural similarity to aquaporins, which along with electrophysiological data suggest a channel-like permeation mechanism. Given the narrow and relatively hydrophobic nature of the permeation pore, the selectivity mechanism of FNTs remains poorly understood. The protonation state of the solutes, as well as the key residues involved in selectivity and gating have been debated, wherein a conserved histidine residue located central in the pore has been proposed to be crucial. We use atomistic molecular dynamics simulations to compute potentials of mean force for full permeation events of certain ionic and neutral solutes across four FNTs (nirC, HSC and two focA channels). Our analyses reveal a high permeation barrier (>75kJ/mol) for ions through the hydrophobic pore of all investigated channels. This barrier is significantly reduced or annihilated when either the central histidine residue, or the permeating solute are protonated. Moreover, we employ QM/MM calculations to investigate the proton exchange between said residue and the permeating solute, thus giving insight into the molecular details of permeation. In summary, the extensive calculations provide a detailed quantitative picture of the energetics of permeation of physiologically relevant solutes through all FNT subtypes with experimentally solved structure.

2218-Pos Board B355

Extracellular Chloride Regulates TMEM16A Gating

Juan A. Contreras-Vite[1], **Silvia Cruz-Rangel**[1], Patricia Pérez-Cornejo[2], H. Criss Hartzell[3], Jorge Arreola[1].
[1]Institute of Physics, Univ. Autonoma de San Luis Potosi, San Luis Potosi, Mexico, [2]School of Medicine, Univ. Autonoma de San Luis Potosi, San Luis Potosi, Mexico, [3]Cell Biology, Emory University School of Medicine, Atlanta, GA, USA.

The gating mechanism of TMEM16A, an essential subunit of the Ca^{2+}-activated Cl^- channel, remains incompletely understood. Gating of TMEM16A channels is complex because elevation of intracellular calcium concentration ($[Ca^{2+}]_i$), strong depolarizations (Vm), and high extracellular Cl or highly permeant anions seem to contribute. In the present work we show that in TMEM16A no gating currents can be detected, however, external Cl ions might play a role in voltage sensing because the kinetics and magnitude of current activation were dependent on $[Cl^-]_e$. The onset and offset of Cl- currents produced by 0.5 s depolarisations of cells bathed in 137 mM $[Cl^-]_e$ followed a mono-exponential behaviour; a subsequent reduction of $[Cl^-]_e$ to 10 mM diminished the conductance ~2-fold. These data suggest a single gating process regulated by extracellular Cl^-. In contrast, when cells were exposed to 137 mM $[Cl^-]_e$ the onset and offset currents elicited by 20s depolarisations exhibited fast and slow kinetics. Activation of the slow component resulted in large onset Cl- currents that did not reach steady-state at the end of the 20s pulse. This behaviour was abolished after activating TMEM16A with 5 µM $[Ca^{2+}]_i$ or after deleting four residues ($_{448}$EAVK$_{451}$) located in the first intracellular loop. Deleting four additional residues ($_{444}$EEEEEAVK$_{451}$) resulted in Cl- currents that decayed at the end of the 20 s depolarization. To explain our data we developed a 12-state gating model assuming that TMEM16A is activated by a direct, Vm-dependent binding of two Ca^{2+} ions. In this model external Cl^- increases channel open probability by promoting stable Vm-dependent Ca^{2+} binding. Our model reproduced the gating behaviour of the TMEM16A currents in response to voltage, Ca^{2+}, and $[Cl^-]_e$. Thus, we conclude that external Cl stabilises Ca binding and promotes two gating modes in TMEM16A. Supported by grant 219949 from CONACyT.

2219-Pos Board B356

Activation of ATP Secretion via Volume-Regulated Anion Channels by Sphingosine-1-Phosphate in Raw Macrophages

Philipp Burow, Manuela Klapperstück, **Fritz Markwardt**.
JB Institute for Physiology, Martin-Luther University Halle, Halle (Saale), Germany.

Whole cell ion currents were recorded in RAW 264.1 mouse macrophage cells by means of the voltage clamp technique in the whole cell configuration. Outwardly rectifying anion currents were activated by sphingosine-1-phosphate (S1P). The current reversal potential was shifted by replacement of extracellular Cl^- by glutamate$^-$ but not when extracellular Na^+ was substituted by $Tris^+$ revealing that the S1P-induced current was mainly carried by anions. Similar currents were induced by hypotonic extracellular solution. The inhibition of the S1P-induced currents by hypertonic extracellular or hypotonic intracellular solution as well as the inhibitory effects of the anion channel blockers NPPB, tamoxifen and glibenclamide indicate that the anion current is mediated by volume-regulated anion channels (VRAC). The S1P effect was blocked by the blocker of the S1P receptor 1 subtype (S1PR1) W123 and by intracellular GDPβS which points to a signalling via S1PR1 and G-proteins. As ccytochalasin D diminished the action of S1P we conclude that the actin cytoskeleton is involved in the stimulation of VRAC. S1P as well as hypotonic extracellular solution induced a secretion of ATP from the macrophages which was blocked in both cases in a similar way by typical VRAC blockers. We suppose that the S1P-induced ATP secretion in macrophages via activation of VRAC constitutes a functional link between sphingolipid and purinergic signalling in essential processes such as inflammation, migration of leukocytes as well as phagocytosis and killing of intracellular bacteria.
This work was supported by the Roux program of the Medical Faculty of the Martin Luther University Halle (FKZ 28/29).

2220-Pos Board B357

Positioning of the First Extracellular Loop of CFTR Has Significant Effects on CFTR Gating

Daniel T. Infield, Guiying Cui, Christopher Kuang, Nael A. McCarty.
Pediatrics, Emory University, Atlanta, GA, USA.

The first extracellular loop (ECL1) of CFTR contains several residues involved in stabilizing the open state of CFTR. D110 is positioned on the side of ECL1 nearest to the CFTR pore, extracellular to several amino acids in the first transmembrane helix important for chloride permeation. In the present study, we utilized cysteine mutagenesis and electrophysiology to observe real time effects of chemical manipulation on D110C-CFTR and on a double mutant of D110C with K892C in ECL4, across the CFTR pore. Via whole Xenopus oocyte TEVC recording, we found that the reducing agent DTT increased the conductance of D110C-CFTR 3.21 +/− 1.16 fold and D110C/K892C-CFTR 13.0 +/−3.5 fold. Treatment of both variants after DTT with Copper (II) Phenanthroline quickly inhibited them to a similar extent (~75%). Single channel recordings without DTT showed that both mutants contain full conductance comparable to WT-CFTR, but significantly decreased mean burst duration. We previously reported modification of D110C/K892C channels with DTT led to increased openings in multichannel patches, without apparent effects on single channel conductance or open burst duration, indicating that DTT likely breaks a closed-state linkage between D110C and K892C. Modification of D110C-CFTR with DTT resulted in an increase in mean burst duration from 154ms to 369ms. Finally, via TEVC, we found that 20uM Cadmium inhibited DTT-treated D110C-CFTR that reversed within 30s of washout, whereas D110C/K892C-CFTR was inhibited irreversibly in the same context. WT, K892C-, D112C/K892C-, and E115C/K892C-CFTR were unaffected by DTT or Cd2+. We interpret our results to indicate the positioning of the pore-facing end of ECL1 is important for CFTR gating, and the more profound effects of DTT and Cd2+ on D110C/K892C-CFTR versus D110C-CFTR may also indicate ECL1 and ECL4 must separate during CFTR channel opening. Support: NIH 5R01DK56481

2221-Pos Board B358

Effects of the Connexin43 CT on Gap Junction Sub-Domain Organization and Myocyte-Fibroblast Interactions in the Injury Border Zone

Emily L. Ongstad[1], Robert G. Gourdie[2].
[1]Clemson University/ VTCRI, Roanoke, VA, USA, [2]VTCRI, Roanoke, VA, USA.

Phosphorylation of connexin43 (Cx43) has been shown to regulate gap junction (GJ) intercellular communication. A Cx43 carboxyl-terminus(CT) mimetic peptide (aCT1) increases GJ size via reduced Cx43/ZO-1 interaction in vitro and increases phosphorylation of Cx43 at aa S368 (Cx43pS368) in vitro and in vivo. We previously reported the effects of aCT1-treatment in a left ventricular (LV) cryoinjury model in vivo (Circ Res 2011;108(6):704-15). At 8-weeks after cryoinjury, histological assessment showed decreases in scar size and increases in collagen fibril uniformity in aCT1-treated hearts versus controls. Interdigitation of collagen and fibroblasts between myocytes in the injury border zone (IBZ) was reduced in treated hearts versus controls. Treated hearts exhibited a decreased propensity for induced arrhythmias ($p<0.02$) and an increase in Cx43pS368 in the IBZ which correlated with a tendency for Cx43 to be maintained at intercalated discs.
To study the mechanistic effect of aCT1 on myocyte-fibroblast interactions via Cx43, we created a 3D heterocellular system to model the injury border zone