homotetramer of TRK molecules, arrayed radially around a central cluster of four single helices (TM7) from each monomer. The postulated central cluster would form a hydrophobic pore demarked by a cationic vestibule at the intracellular surface, an intruding ring of tryptophane side-chains near the middle of the membrane, and another ring of phenylalanine side-chains toward the outer surface. The arrangement would be similar to that demonstrated for a variety of (pentameric) ligand-gated ion channels, and suggests a mechanism of voltage-modulated hydrophobic gating to underlie penetration of anions (along with water).

1456-Pos  Board B366  
Caveolin-3 regulates the Volume-Regulated Anion Channel in Mouse Ventricular Cells  
Shintaro Yamamoto, Satomi Kita, Takuya Iyoda, Toshiki Yamada, Takahiro Iwamoto.

Caveolae are small invaginated microdomains located with a variety of signal transduction molecules on the plasma membrane. Recent reports showed that the knockout mice of a muscle-specific protein caveolin-3 (Cav-3 KO), a principal component of the caveolae in heart, displayed an enlargement of ventricular cells. Volume-regulated outwardly-rectifying anion channel (VRAC) is activated by membrane stretch, and play a significant role in cell volume regulation in cardiac cells. However, it is unknown the properties of VRAC in the enlarged cardiac cells from Cav-3 KO mice. In this study, we examined that VRAC current and the cell volume regulation in freshly isolated single ventricular cells from Cav-3 KO mice (Hagiwara et al. 2000). Whole-cell current recording showed that the degree of VRAC current induced by extracellular hypotonic solution (HYPO) is markedly reduced in the cells from Cav-3 KO mice, compared to that from wild-type (WT) mice. Video-image analysis revealed that the degree of HYPO-induced cell swelling in Cav-3 KO mice is significantly bigger than that in WT mice, and the regulatory volume decrease, which was seen in WT cells after osmotic swelling, is almost lost in cells from Cav-3 KO mice. This result is in parallel with the VRAC inhibition. In contrast, acidic extracellular pH-activated chloride current and extracellular UTP-activated CFTR current were affected less by the deficiency of caveolin-3. The attenuated VRAC current was restored by intracellular application of a VRAC modulator, phosphatidylinositol 3,4,5-trisphosphate (PIP3). These findings suggested that the attenuation of cardiac VRAC current is due to the PIP3 depletion in Cav-3 KO mice.

1457-Pos  Board B367  
Mitochondrial ROS Elicit Volume-Sensitive Chloride Current in Simulated Cardiac Ischemia/Reperfusion Injury  
Wu Deng, Jun Yin, Edward J. Lesniewsky Jr., Clive M. Baumgarten.

Volume-sensitive Cl current (ICl,swell) is activated by osmotic swelling, mechanical stretch and cardiomyopathies, but its role in cardiac isch-emia/reperfusion injury (IR) is unclear. Mock IR was studied in cultured mouse atrial HL-1 myocytes and freshly isolated rabbit ventricular myocytes. After exposure to mock ischemia media (modified Krebs-Henesleit; pH 6.5, 0.9% O2, 0 mM glucose, 37°C) for 4 hr (HL-1) or 45 min (ventricular myocytes), cells were reperfused for 1-5 hr (pH 7.4, 21% O2, 11 mM glucose, 37°C) before making whole-cell recordings under conditions that isolate anion currents; time-matched controls were maintained in the reperfusion solution throughout. In control HL-1 myocytes/reperfusion injury (UR) is unclear. Mock IR was studied in cultured mouse atrial HL-1 myocytes and freshly isolated rabbit ventricular myocytes. After exposure to mock ischemia media (modified Krebs-Henesleit; pH 6.5, 0.9% O2, 0 mM glucose, 37°C) for 4 hr (HL-1) or 45 min (ventricular myocytes), cells were reperfused for 1-5 hr (pH 7.4, 21% O2, 11 mM glucose, 37°C) before making whole-cell recordings under conditions that isolate anion currents; time-matched controls were maintained in the reperfusion solution throughout. In control HL-1 myocytes, ICl,swell was minimal; ICl,swell was strongly activated by 100 μM H2O2 (48.8 ± 6.0 pA/pF), outwardly rectified in both physiologic and symmetrical Cl-, and H2O2-induced ICl,swell was fully blocked by 10 μM DCPIB (102 ± 2%) or 10 μM taminoxfen (104 ± 6%). In contrast, 69 ± 4% and 71 ± 8% of H2O2-inducible outwardly-rectifying ICl,swell already was activated after IR in isosmotic physiologic and symmetrical Cl- solutions, respectively, and was blocked by DCPIB (98 ± 1%) or taminoxfen (99 ± 1%). IR-induced basal ICl,swell was insensitive to both hyperosmotic (1.5T) shrinkage and block of NADPH oxidase ROS production by gp91ds-tat (500 nM), whereas these interventions suppress ICl,swell evoked by swelling, stretch, angiotsin-II and endothelin-1. In contrast, ICl,swell after IR was inhibited 88 ± 4% by the mitochondrial electron transport blocker rotenone (10 μM), which also suppresses osmotic swelling- and endothelin-induced ICl,swell. In rabbit ventricular myocytes, ICl,swell activation and regulation after IR and in time-matched controls recapitulated results in HL-1 cells. These findings suggest that ICl,swell is stimulated by mitochondrial ROS production in IR and may contribute to cardiac dysfunction.

Computational Electrophysiology on Vdac-1 reveals Mechanism of Anion Flux  
Ulrich Zachariae, Robert Schneider, Adam Lange, Bert L. de Groot.

The voltage-gated anion channel VDAC-1 in mitochondrial outer membranes is the principal passageway between mitochondria and the cytoplasm, transferring ATP, the major source of chemical energy in cells. It is also implicated in rupture of the mitochondrial membrane during mitochondria-dependent cell apoptosis.

We used our newly developed method, computational electrophysiology, capable of modelling detailed ion transfer processes under steady flux conditions in molecular dynamics simulations, to study VDAC-1 embedded in lipid bilayers. In particular, we investigated how changes in transmembrane voltage, alterations in membrane state, functionally important mutations, and the intrinsic dynamics of VDAC-1 impact on channel current and how it is related to the surrounding bilayer.

We found that the beta-barrel structure of VDAC-1 is unexpectedly dynamic, owing to the complete lack of a hydrophobic core, and undergoes large elliptic deformations. Strikingly, the N-terminal helix proves to be the most rigid section of the protein and confers stability to the global structure. Its removal results in greatly enhanced fluctuations and can give rise to a partial collapse of the barrel, especially if coupled to induced shock waves in the membrane. In electrophysiology simulations, the partially collapsed states exhibit subconductance behavior and altered ion selectivity.

1459-Pos  Board B369  
Higher Order Structure of TMEM16a/Anoctamin-1 as Compared to Other Chloride-Conducting Channels  
Ghada Fallah, Silvia Detro-Dassen, Ursula Braam, Frederik Rudolph, Fritz Markwardt, Gunther Schmalzing.

The molecular identity of Ca-activated chloride channels (CaCCs) has been known until recently, when three independent studies identified TMEM16A as a protein with the classic CaCC properties. Due to the lack of sequence homology to other channel proteins, the possible oligomeric structure of TMEM16A cannot be inferred by analogy. Here, we used blue native PAGE (BN-PAGE) and chemical cross-linking combined with BN-PAGE and SDS-PAGE to determine the quaternary structure of the mouse TMEM16A (a) and TMEM16A(ac). As positive controls, we co-analyzed chloride-conducting channels of known oligomeric structure such as the glycine-gated homopentameric x1 glycine receptor (GlyR) and the voltage-gated homodimeric chloride channel hCLC-1. All constructs carried a hexahistidyl tag to allow for one-step affinity purification. Two-electrode voltage clamp recordings revealed that N- or C-terminal His-tagging did not affect the TMEM16A(a)-mediated ion currents in Xenopus laevis oocytes. The proteins were purified on a Ni-NTA affinity resin under non-denaturing conditions using digitonin as a mild detergent from both X. laevis oocytes and HEK293 cells. The non-denatured TMEM16A(a) and TMEM16A(ac) migrated in the BN-PAGE gel as a homo-dimer, as judged by comparison with a concatenated TMEM16A(a) homodimer, the x1 GlyR, and the CLC-1. Cross-linking with glutaraldehyde corroborated the homodimeric TMEM16A(a) structure. The homodimeric TMEM16A(a) assembly dissociated into protomers following denaturation with SDS, and non-reducing SDS-PAGE provided no evidence for inter-subunit disulfide bonds. Based on the identical homodimeric assembly of both the ER-resident and the plasma membrane-bound TMEM16A(a) protein, the non-covalent homodimerization must have already occurred in the ER. This observation classifies the TMEM16A(a) channel as a permanent or obligate oligomer, (i.e. a protein that occurs only in the oligomeric state). Together, our data show that the CaCC member TMEM16A shares an obligate homomeric architecture with the hCLC-1 channel.