bleb patches appears to be larger than proposed previously (Honore et al., 2006, PNAS **103**:6859).

1310-Pos Board B154

Rapid And Efficient Co-reconstitution Of Bacterial Mechanosensitive Ion Channels Of Small And Large Conductance Into Liposomes

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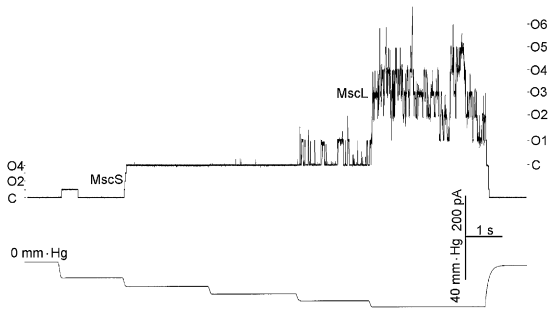
Bacterial mechanosensitive (MS) channels protect bacterial cells from osmotic shock, acting as emergency relief valves (1,2). *E. coli* has three such channels, the MS channel of large conductance (MscL), the MS channel of small conductance (MscS) and the MS channel of mini conductance (MscM). Both MscL and MscS have been extensively studied using the patch-clamp technique in giant spheroplasts (3,4). However, only MscL incorporates efficiently in liposomes (2,5). Here we report the first example of co-reconstitution of both MscS and MscL into azolectin liposomes. We also report reconstitution efficiencies of both proteins into liposomes of different lipid composition and using different incorporation methods.



1. Martinac, B., J. Cell Sci., 117, 2449-2460 (2004).
2. Perozo, E., Nat. Rev. Mol. Biol., 7, 109-119 (2006).
3. Martinac, B., Buechner, M., Delcour, A.H., Adler, J., Kung, C. PNAS, 84, 2297-2301 (1987).
4. Booth, I.R., Edwards, M.D., Black, S., Schumann, U., Miller, S. Nat. Rev. Microbiol., 5, 431-440 (2007).
5. Häse, C.C., Le Dain, A.C., Martinac, B., J Biol. Chem, 270, 18329-18334 (1995).

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1311-Pos Board B155

Modulating The Conductance And Ionic Preference Of MscL, A Biological Nanovalve

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The bacterial mechanosensitive channel of large conductance, MscL, has many properties that are ideal for use as a nanosensor. Previous studies have shown that the pore size is huge (>30Å), it can be translated *in vitro* or synthetically synthesized, and it can spontaneously assemble into a functional complex. In addition, the modality of the channel can be changed; studies have shown that the sensor can be engineered to be sensitive to light, pH, and post-translational chemical modification as well as modulated by different heavy metals and redox. Hence, many studies have suggested its potential in nano-technological applications such as nano-scaled sensors in microchips and drug delivery systems. On the other hand, its large conductance may actually be limiting in some microchip applications, and even though some molecules pass through the MscL nanovalve, modifying its ionic preference could have advantages for vesicular release of charged compounds. Here we demonstrate that MscL can be molecular engineered to have altered conductance or ionic preference to better serve specific purposes. We found that constricting the cytoplasmic loops between the pore and a C-terminal cytoplasmic helical bundle of the channel by bridging cysteines, or coordinating heavy metals with histidines, can decrease the channel conductance as much as 50%; in both instances, the change is reversible. In addition, we found that the ionic preference of the channel can be modified by altering residues near the pore; changing the ionic preference of MscL towards anionic alters the permeability of spermidine, a polycationic organic compound. In summary, our results demonstrate that the conductance and ionic preference of the MscL nanovalve can be modified, and thus designed for specific applications. Keywords: MscL; channel conductance; ionic preference; Nanotechnology.

1312-Pos Board B156

Adaptive Behavior of Bacterial Mechanosensitive Channels in Excised Patches is Coupled to Membrane Mechanics

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MscS, a tension-driven osmolyte release valve residing in the inner membrane of E. coli, exhibits complex adaptive behavior, whereas MscL, its functional counterpart, was considered non-adaptive. When a membrane patch is held under a constant non-saturating pressure gradient, MscS exhibits desensitization (mode-shifting) manifested as a reversible closure followed by complete inactivation. Attempts to utilize MscL as a non-adaptive 'reference' channel revealed that a prolonged exposure of patches to sub-threshold tensions right-shifts activation curves for both MscS and MscL with similar magnitudes and time courses. MscS channels were also found to retain a 'memory' of prior desensitization, returning to the mode-shifted state after being fully opened by a saturating pressure pulse. When recorded in the whole-spheroplast mode under positive pipette pressure, MscS shows no desensitization whereas some inactivation still occurs. We thus link desensitization observed specifically in excised patches with mechanical relaxation of the inner leaflet not attached to the glass pipette, which may create a distribution of tension less favorable for opening. To further characterize and separate the processes of desensitization and inactivation in MscS we applied multi-pulse pressure protocols to excised patches. The results indicated that membrane tension slows reversible closure (desensitization) but speeds up inactivation and strongly impedes the process of recovery from inactivation. These dependencies indicate that the MscS channel contracts in the plane of the membrane when it reversibly desensitizes, but further expands when it inactivates. This calls for models with a more compact gate formed by the TM3 helices in both closed and inactivated states. In contrast, the peripheral transmembrane helices (TM1-TM2) can assume different conformations to confer a larger in-plane area for the inactivated state.

1313-Pos Board B157

Gating Of Bacterial Cyclic Nucleotide Gated (bCNG) Channels In Response To Membrane Tension

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We have identified, cloned, and characterized a new family of bacterial cyclic nucleotide gated (bCNG) ion channels. While we have demonstrated that these channels gate in response to cyclic adenosine monophosphate (cAMP), all bCNG channels exhibit significant homology to the pore lining helix (TM3) of the mechanosensitive channel of small conductance (MscS). This homology suggests that these channels might gate in response to mechanical stress in addition to ligand binding. To test this hypothesis, we have explored the ability of bCNG channels to rescue MscL/MscS/MscK null *E. coli* from osmotic downshock. While some homologues, such as bCNG from *Synechococcus sp. PCC 6803,* show rescue similar to that observed for wild-type *E. coli* MscS, other homologues, such as bCNG from *M. loti,* do not exhibit osmotic rescue. In the bCNG channel family, the numbers of transmembrane domains varies from two to six, with high homology between pore lining helices. Our current data implies that bCNG channels with greater homology to MscS are more likely to be mechanosensitive.

1314-Pos Board B158

Caveolae Act As Membrane Reserves Which May Limit $I_{Cl,swell}$ Activation During Cardiac Myocyte Swelling

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The channel responsible for swelling-activated chloride current ($I_{Cl,swell}$) is a mechanosensor which responds to changes in membrane tension during cell swelling, and regulates cell volume. It has been proposed that the $I_{Cl,swell}$ channel (or elements that regulate this) are dependent on caveolae, and we have previously shown that disrupting caveolae increases the rate of hyposmotic cardiac myocyte swelling. Here we test the hypothesis that the role of caveolae as a membrane reserve limits activation of $I_{Cl,swell}$. Rat ventricular myocytes were treated with methyl- β-cyclodextrin (MBCD) to disrupt caveolae and exposed to 0.02T solution (until cell lysis) or 0.64T solution for 10-15 min (swelling). Maximum cell volume achieved prior to lysis was calculated from a video image. Swollen cells (0.64T) were fixed for electron microscopy, and the negative inotropic response to swelling used as an index of $I_{Cl,swell}$

activation. Following disruption of caveolae, the time to lysis in 0.02T solution was significantly reduced compared with control cells. In cells fixed for EM, caveolae were defined as invaginations or closed subsarcolemmal vesicles with a diameter of ≈50-100 nm. MBCD and 0.064T hyposmotic swelling significantly reduced the total number of caveolae by 75 and 50% respectively. Both 'open' and 'closed' caveolae were reduced by MBCD, but swelling only affected the 'closed' population. The negative inotropic response observed 6 and 10 min after exposure to 0.064T solution was blocked by the $I_{Cl,swell}$ inhibitor DIDS but enhanced by disruption of caveolae. Our data suggest that swelling causes flattening of 'open' caveolae, in tandem with sarcolemmal incorporation of 'closed' caveolae. We propose that disrupting caveolae removes essential membrane reserves, thereby speeding cell swelling in hyposmotic conditions and promoting activation of mechanosensitive $I_{Cl,swell}$ channels.

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1315-Pos Board B159

Effects of Ion and Water Channels Blockers and Uncouplers on the *Dionaea Muscipula* Ellis Trap Closure

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The Venus flytrap (*Dionaea muscipula* Ellis) captures insects with one of the most rapid movements in the plant kingdom. Here we present detailed experiments for comparative study of effects of inhibitors of ion channels, aquaporins, and uncouplers on kinetics of the trap closing stimulated by mechanical or electrical triggering of the trap. Two method of inhibitors phytoextraction were used: (1) two 10 μL drops of channels blockers or uncouplers were placed on the midrib of the trap or (2) addition of 50 mL of inhibitors to the soil. Both methods of inhibitors phytoextraction give the same effects on the kinetics of the trap closing. Ion and water channels blockers such as $HgCl_2$, TEACl, $ZnCl_2$, $BaCl_2$, as well as uncouplers CCCP, FCCP, 2,4-dinitrophenol, and pentachlorophenol decrease speed and increase time of the trap closing [1]. We applied for the evaluation of the mechanism of trap closing our new hydroelastic curvature mechanism, which is based on the assumption that the lobes possess curvature elasticity and are composed of outer and inner hydraulic layers with different hydrostatic pressure. The open state of the trap contains high elastic energy accumulated due to the hydrostatic pressure difference between the hydraulic layers of the lobe. Stimuli open pores connecting the two layers, water rushes from one hydraulic layer to another, and the trap relaxes to the equilibrium configuration corresponding to the closed state. The detailed mechanism of the trap closing is discussed.

[1] A.G. Volkov, K.J. Coopwood, V.S. Markin, Plant Science 175 (2008) 642-649.

1316-Pos Board B160

Fluid Pressure-Gated Cation Channel in Atrial Myocytes

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Regurgitant jets of blood in patients with mitral valve incompetence are known to predispose to atrial fibrillation. To understand cellular basis for the fibrillation induced by the fluid jet, we examined ionic currents induced by a fluid pressure (FP) in rat atrial myocytes. FP was applied by pressurized rapid (~15 dyne/cm^2) puffing of bathing solutions onto whole-cell clamped single atrial myocytes. Puffing (1-s long) of normal external solution produced inward current (I_{FP}) at a resting membrane potential, which was inactivated independently of FP. The current-voltage relationship of I_{FP} showed inward rectification with a reversal potential of ≈-18 mV. Ca^{2+}-free extracellular solution enhanced I_{FP} by ≈7-fold and eliminated the inactivation of I_{FP}. I_{FP} was decreased by extracellular divalent cations with the strongest suppression by Ca^{2+} ($Ca^{2+} > Cd^{2+} > Ni^{2+}$). Removal of extracellular K^+ or Na^+ decreased I_{FP} by ≈46% or ≈35%, respectively. I_{FP} was almost completely suppressed in K^+- and Na^+-free extracellular solution. Increase of extracellular Ca^{2+} concentration to 75 mM enhanced I_{FP}, indicating contribution of Ca^{2+} to I_{FP}. I_{FP} was resistant to the blockade of the stretch-activated channel or Na^+-Ca^{2+} exchanger. Intracellular Ca^{2+} buffering with 4 mM EGTA did not alter the magnitude and inactivation of I_{FP}. I_{FP} was increased to ≈200% immediately after a depletion of Ca^{2+} in the sarcoplasmic reticulum using 10 mM caffeine. Our data provide functional evidence for a novel inwardly rectifying nonselective cation channel in rat atrial myocytes that is gated by fluid pressure. This channel appears to be inactivated by external Ca^{2+}-dependent mechanism and accelerated by depletion of the Ca^{2+} store. The FP-dependent cation influx at resting potential may be a possible mechanism for the blood-jet induced atrial fibrillation in mitral valve incompetence.

Cardiac Electrophysiology I

1317-Pos Board B161

Endothelin-1 Regulates Volume-Sensitive Chloride Current in Rabbit Atrial Myocytes via Reactive Oxygen Species from Mitochondria and NADPH Oxidase

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Angiotensin II (AngII) signaling and reactive oxygen species (ROS) produced by NADPH oxidase (NOX) are implicated in the activation of volume-sensitive Cl current ($I_{Cl,swell}$) by both beta$_1$-integrin stretch and osmotic swelling. Because endothelin-1 (ET-1) is a potential downstream mediator of AngII and ET-1 blockade abrogates AngII-induced ROS generation, we studied how ET-1 signaling regulates $I_{Cl,swell}$. Under isosmotic conditions, ET-1 (10 nM) elicited an outwardly rectifying Cl current that was fully blocked by the highly selective $I_{Cl,swell}$ inhibitor DCPIB (10 μM) and by osmotic shrinkage. Selective ET_A (BQ-123, 1 μM) but not ET_B blockade (BQ-788, 100 nM) fully suppressed ET-1-induced current. ET-1-induced $I_{Cl,swell}$ also was abolished by inhibitors of EGFR kinase (AG1478, 10 μM) and PI-3K (LY294002, 20 μM; wortmannin, 500 nM), which also suppress stretch- and swelling-induced $I_{Cl,swell}$. ERK inhibitors (PD 98059, 10 μM; U0216, 1 μM) partially and fully blocked ET-1- and EGF- induced currents, respectively, but did not effect $I_{Cl,swell}$ elicited by H_2O_2. ET-1 acts downstream from AngII. ET_A blockade (BQ-123) abolished $I_{Cl,swell}$ elicited by both AngII and osmotic swelling, whereas AT_1 blockade (losartan, 5 μM) did not effect ET-1-induced $I_{Cl,swell}$. Both NOX and mitochondria are important sources of ROS in cardiomyocytes. Blocking NOX with apocynin (500 μM) or mitochondrial complex I with rotenone (10 μM) both completely suppressed ET-1-induced $I_{Cl,swell}$. In contrast, $I_{Cl,swell}$ elicited by antimycin A (10 μM), which stimulates superoxide production by mitochondrial complex III, was insensitive to apocynin and the NOX fusion peptide inhibitor gp91ds-tat (500 nM). These data suggest that ET-1 and ET_A receptors are required intermediates in AngII-, swelling-, and stretch-induced activation of $I_{Cl,swell}$. Moreover, enhancement of mitochondrial ROS production by ROS from NOX is likely to contribute to activation of $I_{Cl,swell}$ by ET-1.

1318-Pos Board B162

HIV Protease Inhibitors Activate Volume-Sensitive Chloride Current in Ventricular Myocytes by Generating Mitochondrial Reactive Oxygen Species

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HIV protease inhibitors (HIV PI) have been successfully used to reduce morbidity and mortality of HIV infection. However, their long-term use causes significant side effects including cardiac arrhythmias. Previously we showed that outwardly-rectifying, volume-sensitive Cl current ($I_{Cl,swell}$) is regulated by signaling pathways that elicit production of reactive oxygen species (ROS). Because certain HIV PI recently were reported to augment ROS production, we tested whether HIV PI stimulate $I_{Cl,swell}$ in rabbit ventricular myocytes. Under isosmotic conditions, ritonavir (15 μM, 20 min) and lopinavir (15 μM, 25 min) induced outwardly-rectifying Cl currents (1.5 ± 0.3 pA/pF and 1.9 ± 0.3 pA/pF at +60 mV, respectively) that were fully inhibited by the highly selective $I_{Cl,swell}$-blocker DCPIB (10 μM). In contrast, amprenavir (15 μM, 30 min) and nelfinavir (15 μM, 30 min) did not modulate $I_{Cl,swell}$, and raltegravir (MK-0518, 15 μM, 30 min), an HIV integrase inhibitor, also was ineffective. Two major sources of ROS in cardiomyocytes are sarcolemmal NADPH oxidase and mitochondria. The specific NADPH oxidase inhibitor apocynin (500 μM) failed to inhibit the ritonavir- or lopinavir-induced currents, although we previously found apocynin blocks $I_{Cl,swell}$ activation upon osmotic swelling and stretch. In contrast, rotenone (10 μM, 30 min), a mitochondrial complex I inhibitor that limits electron flux to and ROS production by complex III, blocked 102 ± 4% of ritonavir- and 82 ± 12% of lopinavir-induced $I_{Cl,swell}$. Furthermore, the membrane-permeant, glutathione peroxidase mimetic ebselen (15 μM, 15 min) suppressed $I_{Cl,swell}$ elicited by ritonavir (102 ± 3%) and lopinavir (93 ± 6%). These results suggest that ritonavir and lopinavir activate $I_{Cl,swell}$ via mitochondrial ROS production by complex III. Activation of $I_{Cl,swell}$ by certain HIV PI may contribute to their untoward effects in heart and potentially other tissues.