Platform AD: Mechanosentitive Channels

1001-Plat Modulation of Human Erythrocyte mechano-activated K^+ channel by intracellular Ca^{2+}

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Human Red Blood Cells (hRBC) present a life span of 120 days, and it is widely accepted that the Ca^{2+} content of these cells increases during this period of time. It has been proposed that the increased Ca^{2+} concentration characteristic of senescent cells is produced by mean of mechanical shear at microcirculation level (1,2). Using the Patch Clamp technique we have characterized a K^+ channel after applying step-wise pressure changes to the patched membrane mimicking the hRBC passage through a capillary. This channel presents a sigmoid dependence of Po on applied pressure and a mean conductance of 20pS (140mM KCl, 10mM NaCl, pH7.0) (*HEMKCA: Human Erythrocyte Mechano-activated K channel A)*) (1). Here we present a partial characterization of the complex effect on Po produced by the internal Ca^{2+} concentration; for instance, a rise of Ca^{2+} concentration by 10 times from 10 uM to 100 uM, produce an increase by 20 to 30 times on Po, this effect present no dependence on membrane potential, and is saturated at high Ca^{2+} concentration (aprox. 100uM), interestingly the increase in Po seems not to be related with changes on mean open time. These determination were carried out in the absence of ATP. Hereby, we present new evidence for the HEMKCA, and it is suggested and discusses how these finding strongly suggest that this channel play a key role in the senescence process of hRBC.

1002-Plat Characteristics Of The Stretch-activated Currents In Single Isolated Cardiomyocyte From Pulmonary Vein Of Rabbit

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Atrial fibrillation (AF) is the most prevalent arrhythmia. Pulmonary veins are one of the important ectopic foci for provoking AF. Since the pulmonary vein is much thinner than atrium, it must be more vulnerable to the stretch. For the first time, in this report using single isolated cardiac myocytes of pulmonary vein of rabbit, we would like to examine the characteristics of the stretch activated currents induced by the swelling and the axial mechanical stretch. The swelling induced stretch activated both non-selective cationic current (NSC) and Cl^- current. The swelling induced Cl^- current ($I_{Cl,swell}$) was inhibited by DIDS and the swelling induced NSC ($I_{NSC,swell}$) was inhibited by Gd^{3+}. The cationic selectivity for $I_{NSC,swell}$ was $K^+ > Cs^+ > Na^+ > Li^+$. The activation of $I_{NSC,swell}$ was faster than that of $I_{Cl,swell}$. In the presence of high K^+ in the bath solution, $I_{NSC,swell}$ showed the limited conductance below at -70 mV. The mechanical stretch could induce a Gd^{3+} and streptomycin sensitive NSC right after the mechanical stretch. When the stretch was applied more than 5 mins, the DIDS sensitive current started to be activated. $I_{NSC,stretch}$ showed the higher permeability for K^+ than Na^+. There were some different characteristics between $I_{NSC,swell}$ and $I_{NSC,stretch}$. such as a streptomycin-sensitivity, a limited conductance for K^+, the activation time course. Both the swelling induced stretch and the mechanical stretch could depolarize maximal diastolic potentials and change the shape of the spontaneous action potentials. The stretch induced anion currents and cation currents are functionally existed in the cardiac myocytes in the main pulmonary vein and can have the pathophysiological roles in developing AF in the dilated conditions.

1003-Plat Caveolin-3 Effects On Stretch Channels And Membrane Mechanical Properties In Myotube Membranes

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Caveolin is a cytoplasmically located intermembrane protein that induces membrane curvature and the formation of $\sim$70 nm diameter membrane vesicular-like structures on the membrane surface called caveolae. Caveolae membranes are enriched in cholesterol and signaling proteins (ion channels, receptor mediated kinases and G-proteins). Caveolin-3 is a muscle specific isoform and its expression level may regulate cytoskeletal interaction with the sarcolemma. We have previously shown that patches from mouse myotube membranes have significantly larger capacitance changes when stretched than non-muscle cell types. This may be due to the high levels of caveolae on muscle cell membranes. Further, dystrophic myotubes which overexpress caveolin show a significant increase in membrane viscocity and increased stretch channel activity.

We have over-expressed a GFP-caveolin-3 fusion protein in myotubes and show that the GFP fluorescence is correctly localized to subsarcolemma reticulated structures (Figure 1A). These reticulated structures genesis of the T-tubule system in differentiated myofibers. GFP expressing myotubes have significantly higher levels of stetch channel activity (Figure 1B). We are currently assessing the mechanical differences. Reduced caveolae levels will be assessed by cholesterol depletion and caveolin-3 down-regulation with miRNAs.

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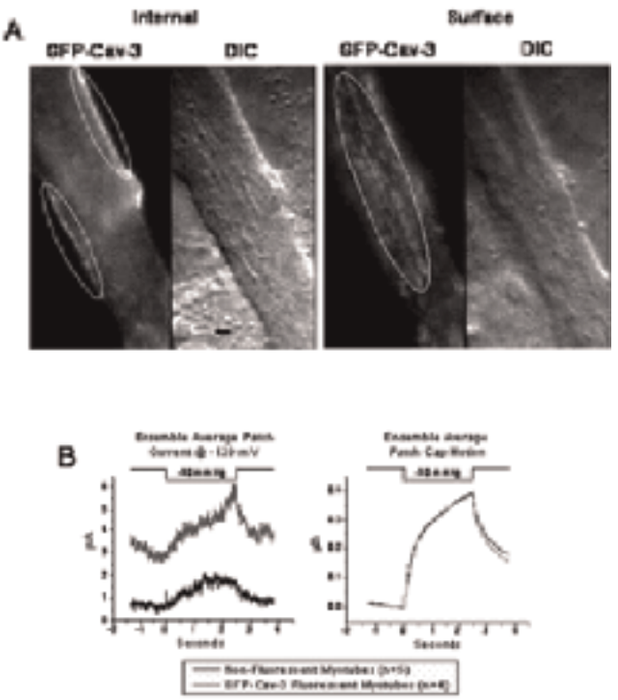

1004-Plat Anionic Phospholipids Control Flux Through The Mechanosensitive Channel MscL

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The mechanosensitive channel of large conductance MscL from *Escherichia coli* is responsible for rescuing the bacterium from hypoosmotic shock. Purified MscL is fully active when reconstituted into model membranes, so that the signal to open the channel must be transduced directly from the lipid molecules to the protein. We have reconstituted MscL into sealed vesicles and the effects of lipid structure on the flux of the fluorescent molecule calcein through the open channel have been studied. Flux through MscL was small when the lipid was phosphatidylcholine but the addition of the anionic lipids phosphatidylglycerol, phosphatidic acid or cardiolipin resulted in large increases in the flux of calcein through the channel. Flux of calcein through MscL reconstituted into mixtures of anionic lipids and phosphatidylethanolamine was less than that observed in mixtures of anionic lipids with phosphatidylcholine. We conclude that the anionic lipids are important for the flux through MscL.

1005-Plat Single Channel Analysis of a Mechanosensitive Channel Reconstituted in a Tethered Lipid Bilayer Membrane Gated by an Applied Voltage

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The functionality of Mechanosensitive (MS) ion channels has been studied in both spheroplasts and liposomes using the patch clamp technique with a pressure applied to create tension in the membrane. Here, *E coli*'s MS channel of large conductance (MscL) was reconstituted into a supported membrane and an external electric field was utilized to create tension. This *novel* approach was made possible using a tethered lipid bilayer membrane (tBLM), which is part of an engineered microelectronic array chip. With the functional and structural knowledge of MscL and the combined flexibility and long-term bilayer stability of the tBLM, analyzing MscL in this system allowed for general fundamental understanding of mechanosensitive channels on solid supports. Single ion channel activity obtained was characteristic for MscL, however, with lower conductivity. MscL was gated using only a transmembrane potential of 300 mV, no pressure across the membrane. This potential should create membrane tension of 12 dynes/cm, which is in agrement with measured values required to gate the channel in both sphereoplasts and proteoliposomes. These findings show the possibility of using the MscL as a release valve for engineered membrane devices; bringing us one step closer to mimicking the true function of the living cell.

1006-Plat Structural Dynamics of MscS Transmembrane Segments in the Open State. A Site-directed Spin-labeling Analysis

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The mechanosensitive channel of small conductance (MscS) is part of a coordinated response to osmotic challenges in *E. coli.* MscS responds to changes in membrane tension by opening a 1.2 nS pore that serves as an osmotic safety valve. Earlier EPR analysis of MscS in the closed conformation showed that in a membrane environment, MscS displays a significant reorientation of the transmembrane helices towards the bilayer normal, forming a more compact structure. Here, we have stabilized the open conformation of MscS by modifying the transmembrane tension profile through the incorporation of cone-shaped amphiphiles. Structural characterization of

the open state was carried out by site-directed spin labeling and EPR spectroscopy of residues encompassing the membrane-embedded regions of the channel in addition to its N-terminal domain (residues 2–131). Purified cysteine mutants were spin labeled and reconstituted into DOPC:POPG vesicles and their X-band CW EPR spectra obtained at room temperature. Conformational changes of the N-terminus and TM segments during MscS gating were evaluated from analysis of spin label mobility and accessibility to O_2 and NiEdda. Additionally, the conformation of residues in the aqueous interface was monitored by collision with DOGS-NTA[Ni(II)] lipids. Our data suggests that transition to the open state is accompanied by an increase in overall dynamics, and involves significant rearrangements of the TM segments. A model for the open state is proposed.

1007-Plat Mechanosensitive Channel Mscs In The Open State: Modeling Of The Transition, Explicit Simulations And Experimental Measurements Of Conductance

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E. coli MscS, a tension-activated osmolyte release valve, shows a non-saturable conductance (1.2 nS in a 39 mS/cm electrolyte) and weak preference for anions. The crystal structure of MscS guided our quest for the transition pathways. We applied a new extrapolated motion protocol (cycles of displacements, minimizations and short simulations) to the previously generated compact resting conformation of MscS with the reconstructed N-termini. We reproducibly observed tilting and straightening of the kinked pore-lining TM3 helices during the barrel expansion. A similar transition was reproduced in all-atom steered MD simulations with tension applied only to the lipid-facing TM1-TM2 helices indicating that the modeled tight TM1-TM2-TM3 association is sufficient for the bilayer-to-gate tension transmission. Extended simulations confirmed the stability of the open conformation with straightened TM3s. An observed 53° rotation of TM3s changed the geometry and polarity of the pore allowing for stable voltage-independent hydration and both cations and anions throughout the whole pore. The resultant open state (1.6 nm pore) satisfied the experimental conductance and in-plane expansion. Applied electric field produced a flow of both K^+ and Cl^-, with Cl^- current dominating at higher transmembrane voltages. The conductance and rectification at hyperpolarizing voltages agreed well with experiments. Electro-osmotic water flux strongly correlated with the chloride current (8 waters per Cl^-). We conclude that

1. the barrel expansion involving tilting, straightening, and rotation of the TM3 helices accounts well for the geometry and conductive properties of the open state;
2. at low voltages ion passage through the pore is similar to electrodiffusion thus macroscopic estimations reasonably approximate the experimental and MD-simulated conductances;
3. increased interaction of the opposing cationic and anionic fluxes may cause stronger selectivity at higher voltages.

1008-Plat Pore Mutations of the *Escherichia coli* MscS Mechanosensitive Channel Affect Desensitisation

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Bacterial mechanosensitive channels react to rapid increases in membrane tension by opening large, non-specific pores in the cytoplasmic membrane with the function of releasing small solutes and ions to halt the instantaneous water influx experienced when cells transfer from a high to low osmolarity environment. Consequently they prevent membrane rupture and cell death. To date, three of the six *Escherichia coli* mechanosensitive channel homologues have been cloned and analysed using patch-clamp electrophysiology. Each channel gates at a different level of pressure and is associated with a specific conductance, however, MscS exhibits a unique characteristic not shared by the other channels: the existence of a desensitised, non-conducting state under sustained pressure. It has been shown that the rate of desensitisation is inversely proportional to the amount of pressure applied, with saturating pressures not allowing entry to the desensitised conformation, but little is known about the mechanism that generates this state. The crystal structure of MscS depicts the pore-lining helix bending sharply about halfway along its length, at Gly113. Using site-directed mutagenesis, we have identified a number of mutations at this hinge location that have a profound effect on the channel desensitising. Substitution with non-polar (Ala; Pro) or polar (Asp; Arg; Ser) residues inhibited desensitisation. Intriguingly, mutation to Met did not obstruct attainment of the desensitised conformation. Thus it appears that although Gly is not specifically required at position 113, MscS desensitisation is strongly influenced by the residue located here. We have also discovered positions further into the pore sequence, 109, 102 and 101, which block transition to the desensitised state with certain substitutions. MscS desensitisation is precisely controlled and further analysis of these pore mutations will help to understand the requirements for the transition into this unique mechanosensitive channel state.

Platform AE: Cardiac Muscle

1009-Plat HCM-linked R403Q Mutant Myosin Motor Directly Alters Cardiac Myocyte Calcium Homeostasis And Contractility

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Mutations in sarcomere proteins cause hypertrophic cardiomyopathy (HCM), an inherited disease characterized by ventricular hypertrophy, arrhythmias and sudden cardiac death. The human β-myosin heavy chain gene (MYH7) is one of the most commonly affected sarcomere components in HCM. The acute effects of human β-myosin heavy chain HCM mutations on cardiac myocyte intracellular calcium homeostasis and contractility, however, are not fully understood. We constructed recombinant adenoviruses to