Board B437

There are limited means to detect the allosteric transitions postulated to occur during CFTR channel gating. While CFTR catalyses the irreversible hydrolysis of its physiological ligand (ATP), there is disagreement about whether CFTR channel gating is a reversible process (Csanady et al., 2006; Aleksandrov et al. 2007). Linear Free Energy Relationship (LFER) analysis which is applicable only to processes exhibiting microscopic reversibility has been successfully applied to ligand gated channels (Grosman et al., 2000). Not only can this approach provide information about conformational motion associated with gating activity but it can also serve as an independent criterion of microscopic reversibility. To test the feasibility of the application of Φ value analysis to CFTR gating, we first analyzed gating of single CFTR channels stimulated with different nucleotide ligands. The log-log graph of opening rate constants versus the equilibrium constants is linear, confirming that CFTR channel gating occurs via reversible pathway. An irreversible mechanism with different pathways for opening and closing should yield a curved rather than a linear relationship. The fact that the Φ value is approximately 1 indicates that all conformational transitions in the binding site are essentially complete before channel opening. Different CFTR structural domains also were tested by LFER analysis. Comparison of the Φ values in each case enabled postulation of a conformational wave propagated from the ligand binding site to the channel pore. This approach can complement other methods of characterizing the dynamic events involved in the allosteric coupling between CFTR's two principal activities.

References

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Mechanosensitive Channels

591-Pos Effect Of High Hydrostatic Pressure And Trimethyl N-oxide On Gating Of The Mechanosensitive Channel Of Small Conductance Of *E. Coli*

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Board B434

The activity of MscS, the bacterial mechanosensitive ion channel of small conductance, has been investigated under high hydrostatic pressure (HHP) using the "flying-patch" patch-clamp technique. In inside-out excised patches of giant spheroplasts of E. coli, MscS was activated by negative pipette voltage to allow for open probability measurement at different levels of HHP up to 90 MPa. MscS open probability was found to gradually decrease on increasing HHP. To determine the extent that the cytoplasmic and transmembrane domains of the channel may contribute to this effect, the osmolyte methylamine N-oxide (TMAO) was applied to the cytoplasmic side of the excised spheroplast membrane patches. In the presence of TMAO the inhibitory effect of HHP on MscS activity was suppressed at pressures of up to 50 MPa. Above 50 MPa, channel open probability decreased similarly in absence or in presence of TMAO indicating that at pressures higher than 50 MPa, TMAO at concentrations used in this study could not counteract the effect of HHP on the MscS channel activity. The change in the reaction volume calculated in the presence of TMAO differs significantly from the reaction volume calculated in absence of TMAO. Our study suggests that TMAO can stabilize the open state of the MscS channel at HHP, most likely by interacting with the cytoplasmic domain of MscS.

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592-Pos Non-channel Function Of Bacterial Mechanosensitive Channel MscS

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Board B435

MscS is an *E.coli* mechanosensitive (MS) channel with homologs found in other bacteria, archea and plants. MS channels in bacteria jettison osmolytes and *E.coli* cells lacking them lyse under osmotic downshocks. MscS is a homoheptamer with a large cytoplasmic domain of unknown function. The domain is mobile upon gating, suggesting that it may undergo state-dependent interaction with binding partners. In effort to identify possible interaction partners, we fused a part of the cytoplasmic region (residues 171–266, $\alpha\beta_3\alpha$ domain) to GST. Using a GST pull-down assay and mass spectrometry we identified FtsZ as a protein that binds specifically to $\alpha\beta_3\alpha$.

FtsZ is a main component of cell division apparatus and forms Z-rings. FtsZ assembly plays an important role in the control of cell division, and its inhibition leads to cell filamentation. Recently, it has been shown that FtsZ is also involved in cell wall synthesis. We found that overexpression of the $\alpha\beta_3\alpha$ domain of MscS, but not of the full-length MscS, results in cell filamentation. Similarly, overexpression of a truncated MscS channel (MscSΔ266) results in cell elongation. MscSΔ266 was previously identified as a channel with impaired inactivation-closure transition (Schumann *et al.*, 2004, FEBS Letters *572*, 233–237). We hypothesize that FtsZ interacts with MscS, via the channel's cytoplasmic domain, and that this interaction occurs selectively in the open or inactivated conformation of the channel. We propose a model in which MscS transiently arrests progression of cell division in osmotically unfavorable conditions.

593-Pos Genetic Screen For A Conducting State Of MscS Mechanosensitive Channel Using K+ Uptake-deficient *Escherichia coli*

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Board B440

MscS is an osmolyte release valve found in most bacteria. MscS is a homoheptamer. A transmembrane region of each subunit consists of peripheral TM1 and TM2 helices and a central TM3 helix, which lines the pore. In the only available structure, which is thought to represent a non-conducting state, TM3 bends at G113 and projects radially out of the central axis where it connects to, and is partially buried by, contiguous β domain. A major effort in the field has been to understand the rearrangement that opens the channel.

In this work, we used a genetic screen that was with success employed earlier for the functional analysis of potassium channels. *E. coli* strains deficient in potassium transport systems (e.g LB2003 or TK2446) cannot grow on media with 1mM potassium, but expression of potassium channels can restore their growth. We have constructed a random mutant library of MscS and screened ~10^7 clones for potassium transport restoration. An analysis of a subset of the clones shows that the most frequent mutations are in the TM3 helix segment between glycines 113 and 121. It had been previously shown that the double mutant G113A/G121A exhibits a severe gain-of-function phenotype and a model of gating was proposed in which opening involves switching from being kinked at one glycine to the other. Our screen suggests that such chronic channel opening can also be induced by negatively charged substitutions on either the inward-facing face of TM3 or on a section of TM3 that is buried by the adjacent β domain. This may suggest that in open state TM3 helix is straight up to G121 and exposed to the pore while the β domain detaches and move away from TM3.

594-Pos Effect Of Gsmtx4 On The Activity Of MscS and MscK Channels Of E. Coli

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Board B441

GsMTx4, a peptide from the spider *Grammostola spatulata*, is an effective and specific inhibitor of stretch-activated mechanosensitive (MS) channels in eukaryotic cells at μM concentrations. Although the structure of this peptide is known, its precise mode of action on the endogenous MSCs and gramicidin remains to be determined. The peptide is thought to interact with membrane lipids via its amphipathic structure, possibly at the boundary between the MS channel proteins and surrounding lipids. GsMTx4 also exerts an antimicrobial effect by inhibiting growth of a number of bacterial species at concentrations of tens of μM. In prokaryotic MS channels, which serve as model systems to explore the basic physical principles of MS channel gating, previous studies have shown that various amphipathic compounds acting at the protein-lipid interface could affect MS channel gating. We have therefore examined the effect of different concentrations of GsMTx4 on MscS and MscK, bacterial MS channels of small conductance, in excised inside-out patches from giant spheroplasts of *E. coli*. At 2 and 4 μM, extracellular GsMTx4 inhibited activation by shifting the gating reaction to higher suction. In contrast, at 12 and 20 μM the peptide facilitated the MS channel opening by increasing the pressure sensitivity. We analysed the reaction kinetics of MscS with and without GsMTx4. The kinetics required a minimum of six states with four different conductance levels and six pressure dependent rate constants. The analysis revealed which of the rates are modified by GsMTx4 and the dimensional changes of MscS at all transitions. Our study shows for the first time that GsMTx4 exhibits concentration dependent effects on bacterial MS channels.

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595-Pos Cytoplasmic Domain of the Bacterial Mechanosensitive Channel MscS Swells up Associated with Channel-Opening

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Board B438

MscS is one of the bacterial mechanosensitive channels directly activated by membrane tension, and forms a homoheptamer composed of an umbrella-shaped transmembrane domain and a Chinese lantern-shaped cytoplasmic domain. It has been suggested that both lateral expansion of the transmembrane domain and swelling up of the cytoplasmic domain are necessary for channel-opening. Recently, our group showed that these two domains interact during the channel-opening. However, there is no experimental evidence for the hypothetical swelling of the cytoplasmic domain. In this study, the structural changes in the morphology of the cytoplasmic domain during channel-opening were monitored with FRET spectroscopy. Three point mutants of MscS were created; a residue located at upper, middle, or lower part of the cytoplasmic domain was substituted for cysteine (A132C, F178C, or L246C, respectively) and labeled randomly with Alexa488 and Alexa568. Each mutant was reconstituted into asolectin liposomes. The FRET efficiency of L246C was lower than that of others, indicating that the distance between Leu-246 residues in adjacent subunits is longer than that of other residue Ala-132 or Phe-178, suggesting that the cytoplasmic domain has pear-shaped morphology in the closed state. By adding lysophosphatidylcholine, a well known activator of MscS, the intensity of Alexa488 increased while that of Alexa568 decreased, indicating that the FRET efficiency decreased. The largest decrease

in the FRET efficiency was observed in F178C mutant. These results suggest that the cytoplasmic domain enlarged as a whole with the largest expansion at the middle level around Phe-178. In summary, FRET analyses suggest that the cytoplasmic domain of MscS undergoes structural changes from pear-shaped to more spherical "Chinese lantern-shaped" morphology when the channel opens. This is the first direct evidence for the swelling of the cytoplasmic domain during channel-opening.

596-Pos MscS Homolog of Chlamydomonas is a Mechanosensitive Channel in the Intracellular Membrane

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Board B439

The bacterial mechanosensitive channel of small conductance, MscS, serves as a safety valve to protect cell from hypoosmotic shock. While MscS is ubiquitous among prokaryotes, the homologues of MscS are also present in the genome of some eukaryotes (A.thaliana, O.sativa, C.reinharditii, S.pombe). However, the channel function of the eukaryotic homologues has not been identified. Here we show that MscS homolog of Chlamydomonas functions as a mechanosensitive channel in intracellular membrane. RT-PCR analysis showed that at least three MscS homologues (MSC1, MSC2, MSC3) are expressed in Chlamydomonas. These homologues have highest homology with MscS in Third transmembrane domain. Phylogenic analysis indicates that the MscS homologues of Chlamydomonas are a different evolutional origin from the MscS homolog (MSL2, MSL3) of Arabidopsis. When MSC1 or MSC3 was expressed heterogeneously in E.coli cell and a negative pressure is applied through patch membrane, the mechano-gated current was elicited. MSC1 was more permeable to anion than to cation and exhibited inactivation at +60mV. These characteristics are similar to these of MscS. The MSC1 channels began to open at 130 mmHg but closed at pressure close to 0 mmHg. This hysterisis is not present in MscS. Antibodies against the carboxyl terminus of MSC1 recognized small spots in the cytoplasm and the chloroplast. These findings suggest that MSC1 responds to mechanical stress in the intracellular membrane.

597-Pos Tryptophan Residues In The Mechanosensitive Channel MscS From *Escherichia coli* And Application Of Tryptophan Fluorescence Spectroscopy

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Board B442

The mechanosensitive channel MscS releases solutes from bacteria experiencing a hypoosmotic shock. Although a crystal structure of a closed form is available, the mechanism of how the change in pressure causes gating is still poorly understood since structural changes upon gating are unknown. Tryptophan residues can be used as sensitive probes to obtain structural information by fluorescence spectroscopy. We created a tryptophan-free mutant of MscS to allow a selective reintroduction of this probe at sites of interest. A functional and assembled mutant MscS was obtained where the three natural tryptophan residues W16, W240 and W251 were exchanged against other aromatic amino acids. However, mutation of the tryptophan residues showed an effect on the function and stability of the protein. Patch-clamp analysis showed that mutation of W16 caused an increase in the pressure threshold for gating, although this residue is far away from the sealing residues L105 and L109. The residues W240 and W251 are important for the complex stability of the homoheptamer: the mutant W240L/W251L was so labile that only a low concentration of MscS could be found in the membrane and purification was not possible. Substitution against phenylalanine rescued this phenotype suggesting a requirement for aromatic groups. These two amino acids are located at the interface between subunits where they could contribute to binding hot spots. Single tryptophan residues have now been introduced into the pore region of the trytophan-free mutant providing us with information on structural changes in the pore region of the channel during gating.

598-Pos Membrane-perturbing Capacity Of Parabens And Their Effects On A Mechanosensitive Channel Directly Correlate With Hydrophobicity

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Board B443

A lipid membrane can serve as an apolar reservoir for hydrophobic and amphipathic drugs. Prior to interacting with their target channel or transporter, lipophilic agents can change the physical properties of the membrane by partitioning into the bilayer, particularly distorting the lateral pressure profile. Esters of hydroxybenzoic acid (parabens) are widely used as food and cosmetics preservatives, but the mechanism of their broad antibacterial action is unknown. We compare the membrane-perturbing action of ethyl, propyl and butyl parabens measured in Langmuir monolayers with their effects on the mechanosensitive channel MscS in situ. Surface tensions of buffers measured at concentrations up to 10^{-3} M suggest negligible surface activity of ethyl paraben, moderate for propyl and the strongest for butyl paraben. Similarly, pressure-area isotherms of monolayers made of the *E. coli* polar lipids indicate barely detectable pressure increase with ethyl paraben, a moderate pressure increase with propyl paraben, and the strongest lateral pressure increase for butyl paraben. In patch-clamp experiments, ethyl, propyl and butyl parabens added in the bath caused progressively stronger right shifts of MscS P_o-pressure curves, ranging between 5% for the ethyl ester to 34% for butyl ester. No spontaneous activation of the channel by any of the parabens was observed.

Concomitant with the midpoint shifts, parabens increased the rate of MscS inactivation by 0.8, 1.25 and 2.3 log units for ethyl, propyl and butyl esters, respectively. Analysis of concentration dependencies of these effects suggests that the less hydrophobic ethyl paraben may affect the process of channel inactivation directly from the solution, whereas more hydrophobic esters concentrate in the membrane first, acting stronger to promote separation of TM2 and TM3 helices leading to inactivation.

599-Pos Spandex Proteins And Membrane Rupture: Mechanosensitive Closed-closed Transitions as a Benign First Line Of Defense Against Osmotic Fluctuations

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Board B444

Lipid bilayers subjected to stress rupture by the nucleation of holes. Embedded in the bilayers of plasma membranes are many proteins that undergo transitions among discrete conformations. Some transitions involve the protein expanding in the membrane plane. A protein with such a closed-closed transition we dub "stochastic spandex". The transition renders the protein mechanosensitive; the probability distribution of conformations depends on the intensity of membrane stretch. A well-known mechanosensitive protein is the bacterial osmotic valve channel, MscL, whose established function is to open a permeation pathway under nearlytic stress, thereby preventing rupture. We wonder if populations of non-permeant spandex proteins might also be useful, providing a first line of defense against bilayer rupture (e.g. during tension fluctuations due to metabolic production of osmolytes) by partially relaxing the tension in the bilayer. Via a computational approach, we investigate the extent to which spandex proteins could affect nucleation of bilayer holes. We have constructed a model that shows such effects. It includes known mechanical properties of lipid bilayers and mechanosensitive proteins. It incorporates bilayer hole nucleation and shape changes of the proteins through statistical processes that depend on membrane tension and the rate at which the tension varies. We used the model to explore a range of biologically relevant parameter values. The effects of protein concentration, protein species mix, protein expansion tensions, are studied as well as the rate of stretching. We found that protein expansion has a negative feedback on itself, and therefore the tensions at which the proteins actually expand depend on protein concentration. The results show some conditions under which spandex proteins enable lipid bilayers to withstand higher tensions, minimizing the need to open osmotic valve channels.

600-Pos Molecular Dynamics Studies of Gating Mechanism of MscL as a Function of Helix Tilt Angle

Wonpil Im

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Board B445

MscL, the mechanosensitive channel of large conductance, forms a homopentameric pore in which each subunit contains two transmembrane (TM) domains (TM1 and TM2) involved in the channel gating, and a C-terminal cytoplasmic helical domain. The crystal structure (PDB ID:2OAR) represents a closed state of the channel, showing a funnel shaped pore with a large opening on the periplasmic side and the narrowest point near the cytoplasm. Assuming that TM helix tilting due to membrane tension drives MscL gating, molecular dynamics simulations have been performed by applying the recently developed helix tilt restraint potential to the TM helices of MscL in a DMPC lipid bilayer. An initial structure of the MscL/DMPC complex without C-terminal helix (Gln110-Asn125) was generated using Membrane Builder in the CHARMM-GUI website (http://www.charmm-gui.org). CTγP (constant temperature, surface tension, and pressure) dynamics was used to allow the system size along the XY axes to vary during the simulation. The P21 image transformation was used to allow the number of lipid molecules in the top and bottom leaflets to vary during the simulations. Three different simulations have been performed by tilting

1. both TM1 and TM2 helices (10 restraints),
2. only TM1 helices (5 restraints), and
3. only TM2 helices (5 restraints).

The tilt angles of TM1 helices was gradually varied from 35° (PDB ID:2OAR) to 65° by 1° and those of TM2 from 33° (PDB ID:2OAR) to 63° by 1°, together or separately. Detailed information on the change of the pore size, molecular interactions, and pressure profile as a function of helix tilt, and thus the possible gating mechanisms of MscL at the atomic level will be presented.

601-Pos The Role of the Periplasmatic Loops in the Opening Mechanism of the Mechanosensitive Channel MscL

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Board B446

Mechanosensitive channels are part of the defense system of bacterial cells against environmental stress. For example, in the event of hypo-osmotic shock, membrane proteins such as the mechanosensitive channel of large conductance (MscL) can open a nanometer size pore to release pressure differences across the plasma membrane. This opening process is accompanied by large structural changes in MscL, where 10 transmembrane helices are believed to give way for a pore formed by 5 of the helices in the center of the protein by an iris-like motion. The role of the transmembrane helices in channel opening is largely understood, but the function of the periplasmic loops is still poorly characterized. To get insight in the role of these extramembraneous segments for the mechanism of mechanosensation, we studied several MscL deletion mutants. In a series of channel constructs we systematically

deleted part of the periplasmic loop between the transmembrane helices TM1 and TM2. The activity of these MscL mutants, examined using patch clamp channel recordings, and implications for the opening mechanism will be discussed.

We gratefully acknowledge the Australian Research Council, the Center for Functional Nanostructures and the Helmholtz Association for their support.

602-Pos Analysis of Gating Mechanism of E-coli Mechanosensitive Channel MscL by Molecular Dynamics Simulations on Wild Type and Mutant Models

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Board B447

The bacterial mechanosensitive channel of large conductance, MscL, is constituted of homopentamer of a subunit with two (outer and inner) transmembrane alpha-helices and gated by tension in the membrane. Molecular dynamics (MD) simulation studies on *E-coli* MscL have been proposed some models for channel opening. However, as these models do not include fine analyses on MscL-lipid interactions, it remains unclear where in the structure of MscL senses membrane tension and how the sensed force induces channel opening. To address this issue, we constructed a model, in which a closed MscL was embedded in a lipid bilayer, and performed MD simulations under tension generated by reducing the lateral pressure in the membrane. The tension gave a pressure profile across the membrane with two peaks near the glycerol moiety of the outer and inner leaflets. It was also found that F78 in the outer helices facing lipid moiety showed strongest interaction with lipids at the portion with strongest tension, thus may work as a tension sensor of MscL. Upon tension F78 was dragged by lipids followed by tilting the rigid outer and inner helices. The neighboring inner helices cross each other in the inner leaflet, forming the most constricted part of ion permeation pore. Upon tension the crossings moved towards c-terminus of each helix to open the pore. We calculated changes in the interaction energy at the crossings during pore opening and obtained values comparable to those in the gating energy experimentally obtained. To evaluate our MD system, we modeled mutants that are known to open easier (G22N) or harder (F78N) than wild type. These mutant models successfully reproduced the experimentally observed behaviors.

603-Pos Mechanosensitive Channels Of Large Conductance From *E. Coli* Cluster In Azolectin Liposomes

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Board B448

The mechanosensitive channel of large conductance (MscL) is readily reconstituted into artificial liposomes and can be unequivocally identified using the patch-clamp recording technique. Channel clustering has important physiological and therapeutic ramifications and has been well documented in physiological systems, a classic example being the acetylcholine receptor at the neuromuscular junction. However, this phenomenon has not been quantified to date in reconstituted systems. We investigated this phenomenon by patching blisters formed from azolectin liposomes containing reconstituted MscL channels at a protein to lipid ratio of 1:1000. Liposome patches were subjected to increasing tension by applying suction to borosilicate micropipettes (resistance 2.8 to 3.4 MΘ, pipette voltage = +30 mV) until all channels within the patch were opened as evidenced by a saturation of the recorded current. We found that the distribution of the number of channels in a total of 72 patches did not follow a binomial distribution suggesting that the channels were not uniformly distributed. We are presently investigating whether this clustering behaviour is a property of the MscL protein or the consequence of the composition of the bilayer, which may lead to the aggregation of the MscL channels and possible cooperative interaction between the channels.

Supported by the Australian Research Council.

604-Pos Effects of Membrane Tension Altering Amphipaths on Mechanosensitive Chloride Channels

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Board B449

Volume-Activated Chloride Channels (VRAC) are responsible for cell volume regulation in many cells. ClC-2 are inwardly rectifying chloride channels critical for cell function, which appear to be sensitive to hypotonic stress and are activated by voltage, H^+ and Cl^- Although the activation mechanism(s) of both channels remain unclear, membrane tension appears to be involved; however, little data is available to support this idea. In this work we used the patch clamp technique to assay the effect of chlorpromazine (CPZ, 100 μM) and trinitrophenol (TNP, 500 μM) on VRAC and ClC-2 channels. These two amphipaths are known to affect membrane tension through preferential intercalation into one leaflet of the bilayer that results in membrane bending. Chloride currents were recorded from HEK-293 cells using TEACl 140 mM on both sides of the membrane. Cationic CPZ applied under isotonic conditions activated a current which resembled VRAC, however, VRAC channels activated by hypotonic shock were inhibited (~95%, n=5) by CPZ in a Vm-independent manner. In contrast, anionic TNP had no effect when applied under isotonic condition or on VRAC activated by hypotonic shock (n=3). Moreover, application of TNP followed by CPZ did not prevent the CPZ blockade (~95%, n=5). The time course and current amplitude of ClC-2 was not altered by CPZ, but closing of the channels at positive voltage was accelerated

and the Vm-dependence of Po was shifted by −17 mV without changing the slope. TNP slowed activation of ClC-2, decreased current amplitude but did not alter the Vm-dependence of Po. Overall, these results do not appear consistent with bilayer couple mechanism observed in other mechanosensitive channels, indicating that CPZ and TNP may affect Cl^- channel activity through direct interaction.

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605-Pos Roles of Fast Motility of Hair Bundles in Avian and Mammalian Ears

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Board B450

The sensitivity of the ear depends on reverse transduction that modulates mechanical stimulation. Here we compare the effectiveness of hair bundle motility called fast adaptation in the mammalian and avian ears. To address this question, we assume:

1. Fast adaptation of the bundles is based on channel re-closure due to Ca entry,
2. viscous loss must be counteracted by the energy generated by fast adaptation, and
3. the mechanical stimulation is a continuous sinusoid with infinitesimal amplitude.

The condition [2] leads to a limiting frequency f_{lim} which is represented by $\beta N F_g^2 \alpha/(2\pi^2\gamma)$, where β is the Boltzmann factor, N the number of tip links, F_g gating force per channel, α phase factor determined by channel kinetics, and γ the damping coefficient. Transition rates of the channel are determined to maximize α in a 4-state model. Values for γ and N are determined based on the morphology (Lim, 1980 for chinchilla; Tilney and Tilney, 1988, Manley et al., 1996 for chicken).

For mammals, our estimate for f_{lim} is 1.2kHz, far lower than their auditory range ($>$ 20kHz).

For birds, the only available value for gating force F_g is 40fN for cultured basal papilla of chicken (Zhao et al., 1996), which leads to a limiting frequency of ~200Hz. However this F_g value is singular, being $<$1/10 of other animals', including mice, bullfrogs, and turtles. A 10-fold increase in F_g results in a 100-fold increase in the limiting frequency, exceeding the auditory range of birds (mostly $<$ 4kHz).

Our results show the significance of gating force in determining the effectiveness of fast motility. They also show that fast adaptation is much less effective in the mammals than in birds.

606-Pos The Location Of Mechanotransduction Channels In The Hair Cell Bundle

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Board B451

The mechanotransduction (MT) channels are the key player in the hearing process. Unlike the transduction channels of other sensory organs, the molecular identity of the hair cell MT channel is still elusive. It is generally agreed that the MT channels are confined to the hair bundle where they are directly or indirectly connected to extracellular filaments called the tip links. However, the exact location and number of the channels remain controversial though some evidence suggests they are present at both ends of the tip link (Denk et al., 1995). Most existing mechanical models of the hair bundle, because of their simplicity, are inappropriate to study this topic. We have used a full 3-D mechanical model of the hair bundle to explore the implications of different MT channel locations. For modeling, we chose a rat outer hair cell bundle from the apical turn of the cochlea. The MT channel was assumed to be activated according to the existing gating spring theory but, new to this study, we implemented stochastic channel gating, and 10-state channel kinetics involving binding of four calcium ions. Three possible MT channel arrangements were tested:

1. two channels at the upper end of the tip link,
2. two channels at the lower end of the tip link,
3. one channel at the upper end and another channel at the lower end of the tip link.

The simulated responses of these three cases were compared.

607-Pos On Mechanosensitive Ionic Vesical Channels: Na-Influence on Electrical and Motor Vesical Activity

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Board 452

Introduction: Caused by insufficient biophysical information vesical normal/patho-physiology is not enough clarified: This leads to low effectivity of *pharmaco-/electro-therapy* in urinary bladder functional disturbances (hyperactivity, incontinence). Discovery of *stretch*-dependent ionic *channels* in vesical myocytes opened a new dimension in *vesical biophysics*, physiology, pathology [lit.]. On Na-importance for mechano-sensitivity will be reported.

Method: Electrical action potentials (intracellular) and motor (isometric/isotonic) activities of vesical **myocytes** and **detrusor (D)** as well as **trigonal (T)** strips were studied in McEwen-solution [lit.].

Results: *Presence of mechano-sensitive* vesical channels is evident by increase of *rate of rise* (9.2±1.0 V/s)/*fall* (1.3±0.5 V/s) of **spikes (S)** as well as transformation of S into **burst-plateaus (BP)** after **stretch** (3–50 mN). *Na-reduction to 50%* (NaCl 130 mM=100%, isoosmotic *Tris*-Cl-replacement) increased S-/BP-frequency (BP-duration not-changed). NaCl-replacement by LiCl transformed S into BP (n=40). After **stretch** *amplitudes* of spontaneous phasic D-*contractions* (**SPC:** 1–5/min) increased over 2-

times: This effect was stronger in Na-deficient solution (Tris). In T-preparations was observed stronger frequency -augmentation of slow tonic T-contractions (**STC:** 0.1–0.5/min) in stretched than in relaxed preparations: STC was transformed into SPC after NaCl-reduction (*80% Tris*-replacement; n=30).

Conclusion: Na^+ participates probably directly or indirectly (Na/Ca-exchange) in ionic mechanisms concerning Ca^{++}-activated K^+-stretch channels by decrease of inactivation of these channels (increase of Na^+-conductance): This way Na^+ could play a role in the complex mechanisms of electrical (S/BP) and motor (SPC/STC) vesical oscillations.**Lit.:**Eur. Biophys. J. ***34***/6:765/2005, ***26***/1:71/1997;J. Biosci. ***24***:142/1999;Biophys. & Mol. Biol. ***65***/1:170/1996;Proc. Int. Un. Physiol. Sci. ***17***:529/1989, ***21***:A585/2005/Faseb J. ***18***/5;Eur. J. Physiol. ***419***:R98/1991, ***443***:334/2002;Br. J. Urol . ***94***:258–9/2004;Urol. ***68***:78/2006, ***70***/3A:232–3/2007.

608-Pos The Effect Of Caveolae Disruption On The Swelling Response Of Single Rat Ventricular Myocytes

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Board B453

In cardiac myocytes, sarcolemmal integration of sub-membrane caveolae has been reported during hyposmotic swelling. Volume sensitive chloride channels (VSCCs) are known to regulate cardiac myocyte volume, and in non-cardiac cells caveolae have been linked with the regulation of VSCCs. We have therefore investigated the interaction of caveolae and VSCCs in the response of cardiac myocytes to hyposmotic swelling. Caveolae in single rat ventricular myocytes were disrupted by exposure to the cholesterol depleting agent methyl- β-cyclodextrin (MBCD, 2mM) at 37 °C for 1h. Disruption was assessed by the translocation of the caveolae protein caveolin-3 (Cav-3) from the buoyant fraction of a discontinuous sucrose gradient following detergent-free extraction. Myocytes were swollen by reducing the osmolarity of the superfusing Tyrode solution from 280 ± 10 mOsm to 180 ± 10 mOsm under isoionic conditions for 10–15 minutes. The amplitude and time course of the swelling response was monitored by video. VSCCs were blocked by exposure to 10μM Tamoxifen (Tam). MBCD treatment caused a significant translocation of Cav-3 from the buoyant fraction (n= 3 pairs) but hyposmotic swelling did not (n = 6 pairs). Neither MBCD treatment nor Tam treatment affected the swelling-induced increase in cell volume (41% ± 2% in 16 control myocytes). However both treatments decreased the time to half maximal swelling (control 3.4 ± 0.3; MDCD 2.6 ± 0.1; Tam 2.6 ± 0.1 min; P< 0.05, 1-way ANOVA; 16–18 cells in each group). The effects of combining MBCD and Tam treatment were not additive. One explanation of our observations is that VSCCs slow the rate of rat myocyte swelling and require intact caveolae to perform this function.

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609-Pos The Location of Mechanotransductive Proteins in Caveolae and their Stretch-dependent Translocation in the Adult Heart

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Board B454

Caveolae, invaginated lipid rafts, have been assigned an important role in mechanotransduction, although direct evidence for this is lacking in the adult heart. In the present study we have investigated the membrane distribution of proteins which are implicated in mechanotransductive signalling under basal conditions and following stretch. A balloon was inserted into the left ventricle (LV) of Langendorff-perfused adult rat hearts. Control hearts were maintained at an end diastolic pressure of 5 mm Hg. Hearts were stretched (10 or 30 min) by inflating the balloon to give ≅ 95% of maximum developed pressure. LVs were fractionated on a discontinuous sucrose gradient following detergent-free Na_2CO_3 extraction. Buoyant fractions (BF; 4–6 of 12) were enriched in caveolin 3 (Cav 3) but excluded non-caveolae markers (β-adaptin). We looked at the distribution of eNOS, NHE1 and TREK-1 which have been implicated in the contractile and electrical response to stretch in the cardiac myocyte. In the absence of stretch, 100% of eNOS and NHE1 was found in BFs, whereas 94 ± 6% of TREK-1 was outside BFs (n=5). When a line was fitted to the relationship between % in BF and time of stretch there was a tendency for Cav 3 and eNOS (R=−0.99) to move from caveolae and for TREK-1 (R=+0.99) to move to caveolae. NHE distribution did not change with stretch (R=−0.23). These data illustrate the dynamic nature of caveolae in response to mechanical stimuli. Because caveolae can modify signalling by concentrating or excluding elements of signal transduction cascades, and because Cav 3 itself can interact to regulate protein activity, translocation of proteins to/from caveolae lends weight to the hypothesis that these microdomains are involved in mechanotransduction in the heart.

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610-Pos Exploring The Adaptive Characteristics Of The Mechanosensitive Channel Of Small Conductance Mscs

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Board B455

E.coli MscS activates readily to sharply applied tensions, but shows reduced population currents when stimuli are applied slowly. This reduction is the result of channel adaptation consisting of two

sequential processes desensitization and inactivation. Here we separate the properties of these two distinct but kinetically entangled processes. Dose-response curves measured with saturating pressure ramps ~ −200 mmHg of varied speed revealed 2 different activation regimes. Ramps >=1s (1–90s) displayed the lowest and stable $P_{1}/_{2}$ consistently near −120 mmHg. With a machine-limited speed of 10ms, we applied shorter ramps (20–500ms), which revealed an increasing shift of $P_{1}/_{2}$ to higher values apparently due to the slow kinetics of MscS opening. Holding the channel population at intermediate pressures was found to drive desensitization and revealed a third regime, a right-shift of dose-response curves by ~25 mmHg. A new double-pulse protocol revealed that desensitization occurs both above and below the channel's opening threshold. Recovery of desensitized channels to the closed state occurs quickly at zero pressure however this process is strongly inhibited by any residual tension. In contrast to desensitization, inactivation of MscS requires opening followed by transition to a desensitized closed state while tension is present. Inactivated channels that recover under low tension re-enter the desensitized state, not the closed state. Opposite tension dependencies of these two processes suggest that desensitization is associated with a reduction of channel in-plane area, whereas inactivation requires additional expansion. Finally, using triangular stimuli we show that MscS displays prominent hysteresis with ~20–30 mmHg rightward shift (lower pressure) during closing. Mutant channels that change the hydrophobic properties of the pore were shown to either abolish or make worse this hysteresis. It this appears that the open probability of MscS depends critically on the channel's tension prehistory.

Muscle Mechanics & Ultrastructure - I

611-Pos Key-Intermediate in Actomyosin Crossbridge-Cycle during Sliding: A Candidate for Pre-Power Stroke Configuration

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Board B456

We are studying the conformational changes of myosin crossbridges during in vitro sliding, utilizing a novel image analysis to quantitatively compare freeze-replica microscopic images with the atomic models in various conformations. We reported that the lever-arm moiety of crossbridges there is mostly kinked to the opposite side of ADP/Vi-bound structure, implying the need for complete revision of conventional tilting-lever-arm hypothesis. Similar reversely-kinked heads were observed in SH1-SH2 crosslinked myosin. We generated a realistic model of that novel structure corresponding to sliding-intermediate by modifying known crystal structures. pPDM-crosslinked (between SH2 and Lys705) species was used as a start, since the EM image of the motor-domain was the closest among reported structures. Gly695, between oppositely-oriented SH1 and 2, is likely to be the hinge, considering the occurrence of chemical-crosslink between them. Assuming rotation around Gly695 with additional one at Gly765, we could generate a new model that explains all the observed images during sliding. We also tried to reconstruct the 3-D structure of SH-crosslinked analogue of the key-intermediate, from a series of classified and averaged freeze-replica images. Tentative result showed comma-shaped body, compatible to the kinked lever-arm structure abundantly observed during in vitro motility. We noticed the presence of small population of crossbridges in the same configuration but attached to actin filament in a different angle. Since the orientation of the motor-domain of that species relative to actin was identical to that in rigor, we assumed that could be a good candidate for the real pre-power stroke primed configuration, ready to convert to the rigor structure by a simple extension of the lever-arm. Assigning that new intermediate as pre-power stroke, primed configuration, as well as weakly-bound one, we can comprehensively explain all the observed images and previous experimental results.

612-Pos Conserved Glycine Mutants of Myosin

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Board B457

Myosin heavy chain has several highly conserved glycines including G362 and G407. G362 is found at the end of a helix-turn-helix segment on the C-loop known to communicate with the ATP binding site. G407 is part of the myopathy loop, imbedded in a cluster of potential heart disease causing mutations. G407 mutated to valine in β-cardiac myosin has been implicated in hypertrophic cardiomyopathy. Two mutant proteins (G362A and G407V) were constructed in smooth muscle HMM. ATPase, actin-activated myosin ATPase, actin binding, and *in vitro* motility assays were preformed to determine the functional significance of their conservation. The mutations did not affect strong actin binding compared to wild type HMM and Ca^{2+} and Mg^{2+} ATPase assays showed minimal differences among the three proteins, however, EDTA ATPase activity was reduced in both mutants. G407V showed a decreased actin-activated ATPase and a corresponding lower actin motility velocity. In contrast, G362A showed a marked increase in both the actin-activated ATPase and the motility velocity.

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613-Pos R777 Modulates Intrinsic Fluorescence Changes in Smooth Muscle Myosin

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Board B458

Tryptophan 512, located in the rigid relay loop, is the principle endogenous tryptophan sensitive to both nucleotide binding and