2924-Pos Pharmacological Characterization Of Nuclear NFAT Translocation In Cardiac Myocytes

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Nuclear localization of NFAT transcription factors activates pre-hypertrophic genes related to cardiac hypertrophic remodeling. Dephosphorylation of cytoplasmic (inactive) NFAT by Calcineurin activates Ca-dependent translocation of NFAT to the nucleus. Although this mechanism is well defined in many cell types, little is known about the pathways that control nuclear translocation of NFAT in cardiac tissue. We analyzed subcellular localizations of NFAT in cat atrial and rabbit ventricular myocytes in response to experimental interventions that interfere with nuclear import and export pathways for NFAT proteins. Myocytes were infected with recombinant adenoviruses encoding for NFAT-GFP fusion proteins (isoforms NFATc1 and NFATc3) and kept in culture (up to 48 h) to allow detectable protein expression. Ca signals were measured in the presence of NFAT-GFP using the Ca-sensitive dye Rhod-2. Myocytes expressing NFAT-GFP showed normal cellular morphology and Ca transients in response to electrical field stimulation on day 2 in culture. We observed isoform-specific differences in non-stimulated myocytes: While NFATc3 was predominantly localized in the cytoplasm, NFATc1 displayed nuclear localization in most of the cells. This localization was sensitive to the level of intracellular Ca ($[Ca]_i$), since incubation in Ca-free extracellular solution or lowering $[Ca]_i$ using BAPTA- AM induced a re-distribution of NFATc1 to the cytoplasm. Nuclear accumulation of NFATc1 could be enhanced by blocking the nuclear export protein Crm1 using Leptomycin B (40 nM). In case of NFATc3 stimulation with neurohumoral stimuli Endothelin-1 (100 nM) and Angiotensin II (1 μM) resulted in activation of NFAT import and enhanced nuclear localization. These data suggest that nuclear localization of NFAT in cardiac myocytes is not only determined by the import-activating intracellular Ca signals, but also controlled by nuclear export pathways. Furthermore, NFATc1 and c3 isoforms have differential activation pathways in the same cell.

2925-Pos Nanomechanical Basis of Selective Gating in the Nuclear Pore Complex

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The intrigue surrounding the nuclear pore complex (NPC) lies in its ability to restrict or promote cargo translocation between the cytoplasm and nucleus i.e. selective gating. A mechanistic understanding of the NPC *modus operandi* necessitates a detailed knowledge of the physical responses of the natively unfolded phenylalanine-glycine (FG)-rich domains (FG-domains) to the biochemical interactions that promote nucleocytoplasmic transport (NCT). Hence, conventional approaches may be powerful in identifying such protein-protein interactions, but provide only marginal assessments of its biophysical foundations.

In our lab, we have developed an interdisciplinary approach to reconcile the dualistic nature of selective gating within the context of FG-domain behavior by correlating the nanomechanical responses of the Nup153 FG-domain (cNup153) to the biochemical interactions that govern NCT. Thus, an important consideration is to replicate more closely the nanoscopic, contextual details of the NPC, instead of inferring their behavior from "top-down" macroscopic views. Specifically, cNup153 tethered to gold nanostructures (designed to mimic the NPC geometry) gave rise to a polymer brush-like entropic barrier. We found that binding interactions with the transport receptor, karyopherin-β1, caused the FG-domains to collapse into compact molecular conformations. This effect was reversed by the action of RanGTP, which returned the FG-domains into a polymer brush-like, entropic barrier conformation. Immunogold-labeling electron microscopy substantiated these findings *in situ* by showing that the FG-domains were also reversibly collapsed *in vivo*. Based on these results, we conceptualize that selective gating consists of a rapid, stochastic flux of collapsing and distending FG-domains owing to the dynamic nature of NCT. In closing, we will demonstrate how the aforementioned principles of nanomechanical selective gating can be applied to the construction of a *de novo* designed synthetic NPC.

Auditory Systems

2926-Pos The Endocochlear Potential Depends Upon Two K^+-diffusion Potentials And An Electrical Barrier In The Stria Vascularis Of The Inner Ear

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The endocochlear potential (EP) of ~+80 mV is essential for audition. The stria vascularis in the cochlear lateral wall is crucial for EP-formation, but its mechanism has remained largely unknown. We used multibarreled electrodes to measure potential, input-resistance and K^+-concentration ($[K^+]$) in each of the compartments of the stria vascularis. The intrastrial space (IS), an extracellular space between the inner layer comprising marginal cells and the outer layer composed of intermediate and basal cells in the stria, had low $[K^+]$, high positive potential and a high input-resistance. It indicates an electrical isolation of IS from neighboring extracellular fluids. Inhibition of either the $Na^+,K^+,2Cl^-$-cotransporter or the Na^+,K^+-ATPase on the basolateral membrane of marginal cells or blocking the K^+ channel on the apical membrane of intermediate cells reduced both the potential of IS (intrastrial potential; ISP) and EP. Inhibiting the K^+-transporters caused $[K^+]$ of IS to increase and intracellular $[K^+]$ of marginal cells to decrease. Blocking the

channel had little effect on [K^+] in either compartment. Calculations of the K^+-equilibrium potential in each compartment suggest that ISP is formed by the K^+-diffusion potential across the apical membrane of intermediate cells, whereas EP is determined not only by ISP but also by the K^+-diffusion potential across the apical membrane of marginal cells. Therefore a combination of transporters and channels are required to maintain the balance of active K^+-transport in the cochlea and the EP. Interference with any one of these elements should cause serious consequences for audition.

2927-Pos Flexoelectic Electromotility of Inner Ear Hair Cell Cilia

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Active movements of hair cell cilia play a vital role in the exquisite sensitivity and frequency selectivity of hearing. The active behavior is dependent upon the molecular machinery that underlies the mechano- electrical transduction (MET) current that enters cilia near their tips and depolarizes the ciliary membrane. Recent data demonstrate that membrane tethers with dimensions similar to hair cell cilia are electromotile and generate tensile forces when depolarized. This effect appears to have flexoelectirc origins, where the curvature-induced electric dipole of the tether membrane interacts with the intracellular voltage to generate a piezoelectric-like force. We hypothesize that this same mechanism is at play in hair cell stereocilia and contributes significantly to fast forces and electrical events observed during MET. To investigate this idea, we formulated a model of the stereocilia from first principles of physics that includes MET current and the membrane-based flexoelectric effect. Key physical parameters include the stereocila dimensions and the flexoelectric coefficient of lipid membranes. Model results are consistent with the hypothesis that the flexoelectic effect contributes significantly to the power-stroke of fast hair bundle motility, negative stiffness, and fast adaptation – events triggered by and dependent upon the MET current. If true, the hypothesis further suggests a role for the staircase architecture of the hair bundle and might also explain the strong correlation between frequency sensitivity and hair bundle length across organs and species.

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2928-Pos Effects of Bilayer Thickness on the Membrane Motor of Outer Hair Cells

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Board B231

Outer hair cells, a class of mechanoreceptor cells in the cochlea, are critical for the sensitivity and frequency-selectivity of the mammalian ear. This property is due to prestin based electromotility that couples movement of electric charge across the membrane with membrane area. The motile activity can be thus monitored not only by the length of these cylinder-shaped cells but also as nonlinear capacitance (NLC). We examined the effect of membrane thickness on electromotility by incorporating phospholipids of various chain lengths using gamma-cyclodextrin. The experiment was performed in the whole-cell mode of patch clamp using channel blockers in both internal and external media. Phospholipid loaded 5mM gamma-cyclodextrin was perfused from a perfusion pipette. The concentration of the lipid was 100 micromoles. We found that short phospholipid PC10:0 induces large positive shifts (>100mV) in the voltage dependence but makes the membrane leaky, leading to breakage. PC12:0 also shifts the voltage dependence positively by more than 100mV without decreasing the membrane resistance. PC14:0, however, induced a positive shift of ~ 15 mV. For PC22:0, the shift was ~30mV. These results demonstrate that electromotility is extremely sensitive to the length of phospholipids in the plasma membrane. We interpret that hydrophobic mismatch is the most likely mechanism for these shifts. Large shifts for short chain PCs can be explained if the motor molecule takes the thinner conformation at more negative membrane potential. Because the volume of the membrane protein must conserve, the thinner conformation would correspond to larger membrane area and contributes to the elongation of the cell. This interpretation is therefore consistent with the area motor model for electromotility.

2929-Pos Membrane Cholesterol Concentration Affects the Lateral Mobility of Outer Hair Cell Plasma Membrane Constituents

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Board B232

Mammalian hearing exhibits exquisite sensitivity and frequency selectivity which is attributed to the unique electromotile properties of outer hair cells (OHCs). The OHC transmembrane protein prestin functions as both a voltage sensor and mechanical motor, converting changes in membrane potential into axial, cellular deformations. Recent studies suggest that manipulations in membrane cholesterol levels shift the membrane microdomain distribution of prestin, modulate prestin oligomerization states, and alter prestin function. Thus, we hypothesize that OHC plasma membrane cholesterol levels may regulate electromotility either through microdomain-mediated mechanisms that cluster or segregate prestin molecules or via alterations in the material properties of the membrane, which in turn affect the resident proteins. Using fluorescence recovery after photobleaching (FRAP) in HEK cells, we show both the immobile fraction (*IF*) and effective diffusion coefficient (*D*) of prestin-mGFP are affected by membrane cholesterol concentration. Depletion of cholesterol with mβCD causes a significant increase in *IF*, but no change in *D*. However, overloading the plasma membrane with cholesterol significantly increases *D* as does serial loading and depletion, regardless of application order. Preliminary work also suggests that lipid lateral mobility varies among the three regions of

the OHC and that diffusion in individual regions is sensitive to alterations in cholesterol concentration. Cumulatively, these results demonstrate the complexity of prestin-membrane interactions and highlight the importance of their inclusion in models of prestin function.

2930-Pos Amphipath-Induced Nanoscale Curvature in the Outer Hair Cell Plasma Membrane

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The outer hair cell is a unique sensory cell capable of transducing changes in transmembrane potential into whole cell deformations. This electromotile process is believed to endow the mammalian auditory system with its enhanced frequency sensitivity and selectivity. At the molecular level, it is manifested through the action of the transmembrane protein prestin, and the accepted electrical signature is a measured nonlinear capacitance (NLC). Several agents are capable of altering the NLC operating range including the amphipathic compounds salicylate, chlorpromazine and trinitrophenol. These compounds are known to preferentially intercalate into one bilayer leaflet altering membrane curvature. Though the mechanism by which these agents modulate NLC is not fully understood, it is reasonable to speculate their effects on membrane architecture play an important role; however, the magnitude of OHC membrane curvature changes is not large enough to visualize using conventional microscopy. As such, we developed an extension of fluorescence polarization microscopy (FPM) suitable for the cylindrical OHC. FPM is capable of measuring the orientation of a fluorophore in the membrane, and here we utilize it to assess the orientation of di-8-ANEPPS in the OHC. Our results indicate that in untreated cells, di-8-ANEPPS orients at 26° with respect to the membrane. Following treatment with salicylate (10 mM), chlorpromazine (0.1 mM), or trinitrophenol (0.1 mM), this orientation shifts 9–15°. These orientations are consistent with nanoscale changes in membrane curvature. The membrane bending induced by trinitrophenol is reversible by the simultaneous application of CPZ; however, the membrane bending induced by saliclyate is not. These results suggest that TNP and CPZ exert their effects on OHC NLC via a purely membrane-mediated effect, whereas saliclyate's interaction with the plasma membrane is more complicated and may result from the compound's direct interaction with prestin.

2931-Pos Limiting Frequency Of the Mammalian Ear Revisited

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The sensitivity and frequency selectivity of the mammalian ear depend on motile activity of outer hair cells (OHCs). Because electromotility of OHC must be responsible for this function, its dependence on the receptor potential makes it subjected to the intrinsic low-pass RC filter of the cell. In an earlier report (Ospeck et al, Biophys J 2003) we attempted to find such a frequency limit by imposing the condition that this motility must counteract viscous drag. The value obtained was about 10 kHz, respectable but still short of covering the mammalian auditory frequency.

Here we examine whether the cochlear microphonics (CM) can overcoming the RC problem (Dallos and Evans, Science 1995) by considering an optimal case. We assume that the receptor current across the plasma membrane generates the receptor potential (RP) and across an external space the CM. Because of the phase difference of ~180 deg, the CM in turn can enhance the receptor potential of more basal OHCs. Those receiver cells with enhanced RP are, in turn, assumed to convert this electrical energy gain into mechanical energy and transmit back to sustain the vibration of the basilar membrane at the site of the maximal amplitude.

We find that the magnitude of the enhancement indeed exceeds that of RP if the receiver cells are fewer than the generator cells and that this effect may lead up to about 2-fold gain in energy. However, we do not find that this effect extends the previous value of 10 kHz for the frequency limit. The reason is that our previous estimation did not consider an external resistance, which is needed to produce the CM but reduces the RP. Therefore another factor, e.g. fast voltage-gated K-currents, would be required for high frequency enhancement.

2932-Pos Two-photon Excited Fluorescence Intensity- and Lifetime-based NADH Imaging to Determine the Metabolic Status of the Cochlea

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Metabolism and mitochondrial dysfunction are thought to be involved in many different hearing disorders including noise induced hearing loss. We have employed two-photon fluorescence imaging of intrinsic mitochondrial reduced nicotinamide adenine dinucleotide (NADH) and flavoprotein (Fp) electron carriers to study the metabolic status of the different cell types in excised yet intact mouse organ of Corti preparations. NADH and oxidized Fp are both fluorescent when two-photon excited by femtosecond pulses of 740-nm light, so the relative NADH and Fp fluorescence intensities are commonly used as a surrogate for the concentration. We have observed complementary variation in the fluorescence signal of these inherent probes in inner and outer hair cells of the organ of Corti over time after excision. By optimizing the culture conditions, the preparation can be stabilized for evaluation periods exceeding one hour, permitting comparison of the metabolic state of hair cells at various locations. Recent studies, however, have shown that intensity based measurements may be error-prone when assessing fluorophores concentration since lifetime shifts can also occur as a result of changes in the ratio of the free to enzyme-bound fluor-

ophores populations that occur during changes in metabolism. To evaluate the impact of these changes, we have recently employed two-photon excited NADH Fluorescence Lifetime Imaging. Treatment with both metabolic uncouplers and inhibitors caused systematic shifts in both the lifetime and the free to bound ratio of NADH. Assessment of metabolic state by the intensity-based and lifetime-based techniques will be compared for both monolayer cultures and the hair cells of the excised organ of Corti.

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Store-operated Ca^{++} Channels

2933-Pos Store-Operated Ca^{2+}-Induced Ca^{2+} Release Amplifies Cytosolic Ca^{2+} Signaling and Prevents Store Refilling in Jurkat T Cells

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The precise control of many T cell functions relies on cytosolic Ca^{2+} dynamics which is shaped by the release of Ca^{2+} from intracellular store and extracellular Ca^{2+} influx via plasmalemmal store-operated Ca^{2+} (SOC) channels. It is presumed that Ca^{2+} influx via SOC channels is required for store refilling. However, using Fura2 Ca^{2+} probe to simultaneously assess the store content and Ca^{2+} dynamics in the cytosol, we demonstrated that in T lymphocytes the store refilling was inhibited in the presence of extracellular Ca^{2+} and SOC channels activated by store depletion with cyclopiazonic acid (CPA), a reversible blocker of the sarco-endoplasmic reticulum Ca^{2+}-ATPase. Pretreating cells with xestospongin C (10 μM) or ryanodine (400 μM), the antagonists of inositol 1,4,5-trisphosphate (IP3R) or ryanodine (RyR) receptors, respectively, facilitated store refilling while reducing cytosolic Ca^{2+} transients associated with SOC channel activation. These data indicate that Ca^{2+}-induced Ca^{2+} release (CICR) is an essential source of Ca^{2+} for elevation of cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$) upon stimulation. Consistently, inhibition of IP3R or RyR significantly inhibited T cell proliferation and IL2 production. Finally, we observed that store refilling persisted when store-operated Ca^{2+} entry (SOCE) was completely inhibited by 1 μM extracellular La^{3+} (IC_{50} ~ 30 nM), confirming that store can be refilled in the absence of SOCE and global $[Ca^{2+}]_i$ elevation. Store refilling was inhibited by higher $[La^{3+}]$ (IC_{50} ~ 5 μM) suggesting the existence of a store-independent store replenishing pathway that may sustain CICR. We conclude that CICR is essential mechanism regulating $[Ca^{2+}]_I$ dynamics in T cells and that IP3R and/or RyR may represent novel pharmacological targets for manipulation of Ca^{2+}-dependent functions of T lymphocytes.

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2934-Pos

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WITHDRAWN

2935-Pos Different STIM to Orai1 ignaling pathways between *C. elegans* and human

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Endoplasmic reticulum (ER) Ca^{2+} depletion evokes Ca^{2+} entry through Ca^{2+} release activated Ca^{2+} (CRAC) channel in the plasma membrane (PM). Recent studies have identified STIM1 and Orai1 as essential components of CRAC channel. The molecular mechanism by which STIM1 is translocated and targeted on the plasma membrane (PM) and convey signals from the ER lumen to PM is not yet understood. Here we report that in HEK293 cells the nematode *Caenorhabditis elegans* STIM1 (C.STIM1) are pre-oligomerized in puncta at the cell periphery before store depletion, and this oligomization of STIM1 is not sufficient for the aggregation of Orai1 on the opposing PM and CRAC activation. Therefore, C.STIM1 does not have the store-depletion induced translocation steps of human STIM1 (H.STIM1). In contrast, the C.STIM1 puncta and C. elegans Orai1 functioned as a pair that can locally respond to ER store depletion and lead to activation of CRAC channel. By switching the N- and C- termini of C.STIM1 to H.STIM1, we prove that the STIM1 C-terminus decides its resting localization in different organisms. A mutant H.STIM1 lacking the C terminal proline-rich domain and the polybasic sequence motif (PPK) that are absent in C. STIM1 shows the same distribution as C.STIM1 and colocalized with C.STIM1 near the PM before store depletion, proving that targeting STIM1 on the PM is dispensable of PPK domain. Taken together, our results suggest that: 1) *C. elegans* uses a different signaling pathway of STIM1 compared with human, but uses conserved targeting machinery in the C-terminus to trap STIM1 to the cell periphery. 2) The mammalian C-terminal PPK domain evolves as an autoinhibitory mechanism by which STIM1 conformation changes after Ca^{2+} store depletion and exposes the PM-targeting motif for its targeting on the PM.

2936-Pos Functional Comparison Of ORAI Homologs In Regulating Mammalian CRAC Channel Activity

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