

Auditory Systems

425-Pos Board B225

Steady State Deflections Reveal New Bundle Dynamics of Hair Cells

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Hair cells of the inner ear are the mechano-electrical transducers in the biological sound detection process. In an in-vitro preparation of the bullfrog sacculus hair cell bundles exhibit spontaneous oscillations when transepithelial ionic gradients are maintained and the bundles have been decoupled via the removal of the overlying otolithic membrane. These oscillations have been described by a system of nonlinear differential equations whose solutions undergo a bifurcation from a quiescent to an oscillatory state. In our experiments, we find that applying a slow displacement to the bundle tip during spontaneous oscillation leads to a qualitative change in the oscillation profile, implying the crossing of a second bifurcation. We further observe that application of a small steady-state displacement in the direction of tallest stereocilia can enhance bundle sensitivity. We compare these results to offset position under more natural conditions, where the bundles are still coupled to the otolithic membrane.

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Prediction of the Effect of Adaptation and Active HB Mechanics on Prestin-Based Amplification Using a Macroscopic Model of the Cochlea

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The identity of the mammalian cochlear amplifier is a highly debated issue in auditory mechanics. Two different active processes could underlie cochlear amplification: somatic motility and hair bundle (HB) motility. We use a mathematical model of the cochlea to investigate the role of somatic motility and active HB dynamics. The HB is modeled by a nonlinear six-state channel with a calcium binding event responsible for fast adaptation of the transduction and active HB force generation. The dynamics of the HB are linearized for small harmonic perturbation around the operating point and implemented in a macroscopic model of the cochlea that includes feedback from outer hair cell somatic motility. The simulations of the response of the cochlea to low intensity acoustic stimulation show that somatic motility and not HB motility can modulate the BM motion. However the effect of fast adaptation on the transduction channel is a reduction of the sensitivity of the channel to HB deflection that could serve to control the energy delivered by somatic motility and thereby the gain of the BM to low intensity acoustic stimulation.

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The Active Process in Coupled Hair Cells in the Frog Sacculus

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Non-mammalian auditory organs lack outer hair cells yet show characteristic signs of an active process. Mechanically decoupled hair bundles in the frog sacculus have been shown to amplify on a cycle-by-cycle basis and exhibit spontaneous oscillations in vitro.

Although hair bundles do not oscillate spontaneously when the otolithic membrane left intact, we observe other signs of the active process under these native loading conditions. In particular, hair bundles exhibit a biphasic response, similar to the twitch seen in individual cells, when the otolithic membrane is stimulated by a pulse train. When deflected in the excitatory direction by a sinusoidal pulse, hair bundles first follow the stimulus but show significant up to 10 nm - motion in the inhibitory direction and then return to their resting position with a time constant on the order of a millisecond.

We have measured the strength of the mechanical coupling between the hair bundles and the otolithic membrane. We find that the coupling is primarily elastic rather than viscous with an elastic strength that can be up to an order of magnitude times larger than the hair-bundle stiffness. We hypothesize that in addition to providing passive mechanical coupling between hair bundles, the elastic load of the otolithic membrane maintains the hair cells in a quiescent but active regime.

Calcium Signaling Pathways

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Metabolically Induced Cyclic-Amp Oscillations in Pancreatic Beta Cells

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Glucose-induced oscillations in insulin have been shown to be related to oscillations in calcium as well as cyclic-adenosine monophosphate (cAMP). Calcium is a well-known trigger for insulin secretion, and cAMP has been shown to amplify the insulin release. However, the interplay between calcium and cAMP is still being elucidated. For example, ATP, which is the substrate for cAMP production, is also the signal to close KATP channels, depolarizing the membrane and increasing the influx of calcium. And calcium activates both cAMP produc-

tion via adenylyl cyclase and degradation through phosphodiesterase. Furthermore, the substrate for cAMP, ATP, is the direct output of metabolism of glucose. We use a recent mathematical model that includes equations for cellular electrical activity, calcium dynamics, metabolism, cAMP, and insulin secretion to analyze the relationship between these different cellular components. In particular, we show that our model can reproduce recent experiments on the phase relation between calcium oscillations and cAMP. Further, we predict that given (a) the persistence of metabolic, calcium, and cAMP oscillations in stimulatory glucose (11.1mM) and (b) subsequent loss of oscillations in K⁺-channel opener diazoxide then (c) depolarizing the cells with KCl will recover the metabolic and cAMP oscillations while elevating calcium to a fixed, non-oscillatory level. We conclude that oscillations in cAMP and insulin secretion can occur in the absence of oscillations in the cytosolic calcium concentration, although the latter greatly facilitate the former.

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Nad(P)H Oxidase Regulate Glut4 Traffic by Intracellular Calcium Release in Insulin Stimulated L6-Glut4myc Skeletal Muscle Cell

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Insulin-regulated GLUT4 traffic have been widely explored, but the role of calcium release and ROS signaling in this process is poorly understood. We studied insulin-induced GLUT4 traffic in stable expressing GLUT4myc skeletal muscle cells using single cell imaging and quantification of extracellular myc exposure. Exofacial exposure of myc epitope, glucose uptake and Akt^{S473} activation induced by insulin were independent of extracellular calcium. Pre-incubation with the intracellular calcium chelator BAPTA inhibited activation of Akt, p70S6K and ERK1 but not ERK2. On the other hand, p-ERK1/2 was inhibited by 2-APB, an IP₃R inhibitor. The intracellular calcium chelators BAPTA and NES-PV-DsRed (cytosol directed parvalbumin) respectively reduced insulin-dependent extracellular exposure of myc. Moreover, 50μM ryanodine and 20 μM 2-APB partially inhibited the exofacial exposure of myc with an additive effect. In myotubes normal media, the calcium ionophore ionomycin, induced an increase in extracellular exposure of myc and was additive to insulin. In contrast, in myotubes maintained in calcium-free media, the effect of insulin was totally inhibited by ionomycin. Insulin increased H₂O₂ production measured with the cytosolic molecular sensor HyPer and this effect was inhibited by NAC pre-treatment. p47 subunit of NADP(H)ox labeled differentiated myotubes with a striated pattern but was absent in myoblasts. Both antioxidant agents, NAC and trolox partly reduced the externalization of myc as did the NADP(H)ox inhibitor apocynin. These data suggest that insulin induces an increase in NADP(H)ox activity and activation by ROS of RyR and IP₃R intracellular calcium channels to foster GLUT4 translocation. FONDAP 15010006, FONDECYT 3110170.

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Ip3-Dependent Calcium Transients Differentially Modulate Nf-Kappa B Activity in Wild Type and Mdx Myotubes

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In normal skeletal myotubes, tetanic electrical stimulation activates NFκB through intracellular calcium increase released by inositol 1,4,5 triphosphate receptor (IP₃R) and by Ryanodine receptors (RyR). In this work, we studied the differences in calcium transients evoked by electrical stimuli and NFκB activation in wild type (C57) and in mdx (animal model of *Duchenne* muscular dystrophy, DMD) myotubes.

Myoblasts were obtained from C57 or mdx neonatal mice (1-3 days). Myotubes were loaded with fluo-3AM and stimulated with 250 pulses of 0.5 ms duration at 20 Hz and the calcium signal was assessed by fluorescence microscopy. NFκB activation was determined by luciferase reporter assay, p65 immunofluorescence and p65-GFP translocation.

Fast calcium signal (RyR dependent) was significantly reduced in electrically stimulated mdx compared to wild type C57 myotubes (0.62 vs 1.41 RLU, p<0.01). However, the amplitude and kinetics of (IP₃ dependent) slow calcium signals were similar in mdx and C57 myotubes. Electrical stimulation increased the NFκB luciferase activity in normal but not in mdx myotubes. By immunofluorescence, p65-GFP translocation studies and reporter assays we determined that the basal activity of this transcription factor is up-regulated in mdx myotubes and this could be blocked by inhibitors of IP₃-related pathways (100 μM suramine, 10 μM BAPTA-AM, 5 μM xestospongine B) but not by ryanodine (30 μM). We propose that NFκB up regulation in mdx myotubes is modulated by intracellular calcium release through IP₃R. The differences in NFκB activation between normal and dystrophic muscle cells may help to understand the mechanisms of pro-inflammatory cytokines gene expression in *Duchenne* muscular dystrophy.

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