a high-speed complementary metal oxide semiconductor camera to track the movements of hundreds of cells in parallel from dozens of preparations. This work revealed that the spontaneous oscillations exhibit multiple timescales with a slow modulation on a rapid oscillation. Experiments inhibiting the electrical resonance in the cell body show a strong effect on the mechanical oscillations of the hair bundles. This indicates that the electrical oscillation is coupled with the mechanical oscillations of the hair bundles.

2636-Pos

Effect of Having Two Identical Channels Per Tip-Link in Hair Bundles

Bora Sul, Kuni H. Iwasa.
National Institutes of Health, Rockville, MD, USA.

Gating of mechanoelectrical transducer (MET) channels in hair bundles of hair cells is the key step of mechanosensitivity, including sensing sound and balance. Transducer current, gating compliance, which can lead to negative stiffness of the hair bundle, and adaptation have been successfully described by theoretical models that assume that a single MET channel is connected to each tip-link.

However, recent experimental reports have demonstrated that each tip-link is associated with more than one channel. This discrepancy begs an answer to the question: What are the consequences of two MET channels being associated with a single tip-link?

To address this question, we assume that two channels associated with each tip-link are identical and examine the effect of two basic connectivities, series and parallel, of the channels on their gating. We found that the connectivity has much larger effect on gating compliance than on transducer current. With series connection, gating compliance has a single maximum only if it is below a critical value. Further increase of gating compliance splits its maximum into two. Thus negative stiffness, the result of a further increase in gating compliance, appears in two regions on the displacement axis. In contrast, parallel connection leads to gating compliance similar to that predicted by the previous model that assumes a single channel is associated with each tip-link. Although some cooperative effect of the two channels is present, it cannot be significant for sensitive hair bundles. For those reasons, parallel connection of two identical channels is consistent with experimental observations.

2637-Pos

Structural Determinants of Cadherin-23 Function in Hearing and Deafness

Marcos M. Sotomayor[1], Wilhelm Weihofen[2], Rachelle Gaudet[2], David P. Corey[1].
[1]Harvard Medical School / HHMI, Boston, MA, USA, [2]MCB Harvard University, Boston, MA, USA.

The tip link is an essential component of the hair-cell transduction apparatus and has been proposed to be part of a biophysically defined "gating spring". Cadherin-23 and protocadherin-15 likely form the tip link; both proteins belong to the cadherin superfamily of proteins that mediate calcium dependent cell-cell adhesion and are involved in hereditary deafness, yet their molecular structures and elasticity are unknown. Here we present crystal structures for cadherin-23 repeats 1 and 2 (EC1+2). Overall, the structures show a typical cadherin fold for both repeats, but reveal an elongated N-terminus that impairs classical cadherin-cadherin interactions and facilitates the formation of an additional calcium binding site. The linker region between the repeats closely resembles a classical cadherin calcium-binding motif and contains the site of the D124G mutation causing non-syndromic deafness (DFNB12). The crystal structure of EC1+2 carrying this mutation displays a different angle between the repeats than the wild-type protein. Molecular dynamics simulations of wild type structures and various mutant models suggest that deafness mutations and removal of calcium ions control cadherin inter-repeat motion and unfolding strength of hair-cell tip links. The structures along with simulations indicate that cadherin repeats forming the tip link are too stiff to be the gating spring. In addition, the new structures define a previously uncharacterized family of cadherin proteins and begin to suggest mechanisms underlying disease as well as ways in which cadherin-23 may bind end-to-end with itself and also with protocadherin-15 to form the tip link.

Ryanodine Receptors II

2638-Pos

Emerging of Ryanodin Receptors Mediated Calium Signaling Synchroonizes Early Cardiac Contractility

Serge Viatchenko-Karpinski, Sandor Györke.
Ohio Sate University Medical Center, Columbus, OH, USA.

Stem cell transplantation is a promising technique to improve cardiac function. However arrhythmias in regenerating areas are a major concern surrounding the use of cellular cardiomyoplasty. We associate these arrhythmias with developmental changes in intracellular calcium (Ca^{2+}) signaling. Cellular contractility in early cardiac development induced by Ca^{2+} oscillations evoked by Ca^{2+} release from Inositol 1,4,5-triphosphate receptors (IP3Rs), whereas in adult myocytes Ca^{2+} released from the ryanodine receptors (RyR2s) is the main source of cytosolic Ca^{2+} elevation. Recently we have shown that cardiac specific RyR2s mediated Ca^{2+} signaling in early cardiogeneses does exist in early cardiogenesis but functionally silent due to the lack of sufficient luminal Ca^{2+} reserve. Adenovirus mediated calsequestrin (CASQ2) gene transfer in mouse embryonic stem cells potentiated expression of CASQ2 protein in early cardiac development and contributed to the emergence of RyR2s mediated Ca^{2+} signaling in the developing cardiac myocytes. Expression of CASQ2 significantly increased synchronization contractility of early stage (7+1days after in vitro induction of cardiac differentiation from mouse embryonic stem cells cardiac myocytes) in a single cluster of contracted cells (94% of clusters contracted synchronously vs 14% in control). Depletion of RyR2s associated Ca^{2+} stores with caffeine demolished synchronization and reduced frequency of cellular contractility (31 ± 3 vs 48 ± 5 bpm in control). Similar changes were observed in 7+7 differentiation stage cardiomyocytes differentiated from wild type ES cells. Caffeine reduced number of synchronously contracted clusters from 84% to 28%. Frequency of cellular contractility was also reduced from 46 ± 3 to 34 ± 4 bpm.

We conclude that establishment of RyR2s mediated Ca^{2+} signaling in early cardiogenesis is important for synchronization of early cardiac contractility. Ectopic expression of CASQ2 in early cardiogenesis leads to creation of functional RyR2 Ca^{2+} stores and has major impact on stabilization of intercellular contractility.

2639-Pos

Calcium Influx Analysis by TIRF Microscopy on Cultured Primary Myotubes from Patients with *RyR1* Mutations

Susan Treves[1,2], Mirko Vukcevic[1], Thierry Girard[1], Soledad Levano[1], Albert Urwyler[1], Pierre-Yves Jeannet[3], **Francesco Zorzato**[2,1].
[1]Departments of Anaesthesia and Biomedizin, Basel University Hospital, Basel, Switzerland, [2]University of Ferrara, Ferrara, Italy, [3]Unité de Neuropédiatrie CHUV, Lausanne, Switzerland.

Central core disease (CCD) and malignant hyperthermia (MH) have been linked to point mutations in the gene encoding the skeletal muscle sarcoplasmic reticulum calcium release channel (ryanodine receptor), which is localized on human chromosome 19 (*RYR11*). Central core disease is a relatively mild, slowly progressive autosomal dominant myopathy, characterized histologically by the presence of centrally located cores running the length of the muscle fibres. MH is a pharmacogenetic induced hypermetabolic disease. CCD linked RyR1 mutations are associated with depletion of thapsigarin-sensitive stores and to an increase of the resting calcium level. Influx of Ca^{2+} from the extracellular environment is a major factor which influences the level of resting cellular [Ca^{2+}]. Our working hypothesis is that decrease of sarco(endo)plasmic reticulum Ca^{2+} load via leaky ryanodine receptor channels and/or alteration of calcium influx via store operated channels or excitation-coupled Ca^{2+} entry (ECCE), may account for, at least in part, the phenotype of patients with CCD, including muscle weakness and abnormal secretion of inflammatory cytokines from muscle cells and cells of the immune system. We set out to test the validity of our hypothesis by directly investigating the mechanisms activating calcium influx in myotubes from normal individuals and from patients with CCD and MH by TIRF microscopy. Our data shows that mutations in the RyR1 affect ECCE in human myotubes from CCD and MH patients. *This work as supported by grants from A.F.M., S.N.F., Telethon Italy*

2640-Pos

Dysregulation of Ca^{2+} Entry and SR Calcium Leak are Responsible for Elevated Resting Free Ca^{2+} in Triadin-Null Myotubes

Jose M. Eltit[1], **Jose R. Lopez**[1], Wei Feng[2], Isaac N. Pessah[2], P.D. Allen[1], Claudio F. Perez[1].
[1]Brigham and Women's Hospital, Bosotn, MA, USA, [2]University of California, Davis, CA, USA.

Previously we have shown that lack of triadins expression in skeletal muscle cells results in significant increase of myoplasmic resting free Ca^{2+} ($[Ca^{2+}]_r$), suggesting a role for triadins in modulating global intracellular Ca^{2+} homeostasis. To further characterize this mechanism, here we study the effect of triadin on Ca^{2+} entry, $[Ca^{2+}]_r$, and Ca^{2+} release pathways using Mn^{2+} quench, Ca^{2+} selective microelectrodes and Ca^{2+} imaging in Wt and triadin-null myotubes. Comparison of Mn^{2+} quench rates in resting conditions revealed that triadin-null cells have higher Ca^{2+} entry rates than Wt cells. However, prevention of Ca^{2+} entry with Cd^{2+} and La^{3+} was insufficient to restore