View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by Elsevier - Publisher Connector

of force alternans to peak force was at least tenfold larger than the ratio of AP phase II voltage alternans to maximum AP phase II voltage. In conclusion, electrical and mechanical alternans are both rate-dependent and linked via abnormal calcium handling, but mechanical alternans has the greatest amplitude across all pacing rates. Thus, mechanical alternans, due to their greater SNR, may be better predictors for arrhythmogenic propensity in heart failure patients than electrical alternans.

2543-Plat

Na^+/H^+ Exchange Blockers Reveal the Existence of a Skeletal Muscle Ca^{2+}/H^+ Exchanger, which is Altered in Malignant Hyperthermia Muscle Cells

Gaelle Robin[1], Francisco Altamirano[1], Eric Esteve[2], Isaac N. Pessah[1], Paul D. Allen[1], Jose R. Lopez[1].
[1]Molecular Biosciences, University of California at Davis, Davis, CA, USA, [2]Laboratoire HP2, INSERM U1042, Institut Jean Roget, BP170, Grenoble, France.

In resting skeletal muscle fibers intracellular pH (pHi) is kept constant at a relatively alkaline level. The transporters involved in maintaining muscle pHi at rest are the Na^+/H+ exchange system (NHE), and to a lesser extent the Na^+- and Cl–dependent bicarbonate dependent transport systems. Many studies conducted in nerve and smooth cells have suggested a link between changes in intracellular free Ca^{2+} ($[Ca^{2+}]i$) and changes in intracellular pH (pHi) suggesting a Ca^{2+}/H+ exchange (Daugirdas et al, 1995). Malignant hyperthermia (MH) is a pharmacogenetic disorder of skeletal muscle triggered by inhalational anesthetics and depolarizing neuromuscular blocking agents. We have measured $[Ca^{2+}]i$ and pHi simultaneously in Wt and heterozygous R163C myotubes using double barreled Ca^{2+}-selective and single barreled pH+-selective microelectrodes. MH cells have a higher $[Ca^{2+}]i$ and a lower pHi than Wt cells. Treatment of both MH and Wt cells with the NHE inhibitors dimethylamiloride (DMA) or cariporide, caused an increase in $[Ca^{2+}]i$ and a decrease in pHi in a dose dependent manner. These effects were more prominent in MH than Wt myotubes. YM-244769, a high affinity reverse mode NCX3 blocker, did not modify $[Ca^{2+}]i$ overload or the drop in pHi elicited by DMA or cariporide in either cell type, suggesting that the change in $[Ca^{2+}]i$ was not the consequence to an activation of the reverse form of the exchanger. Gd3+ and dantrolene respectively were able to partially inhibit or fully reverse the DMA or cariporide-mediated elevation of $[Ca^{2+}]i$ and acidification in both types of cells. These results suggest the existence of a Ca^{2+}/H+ exchange in skeletal myotubes, which appears to be altered in MH muscle cells.

2544-Plat

Calcium Sparklets in Intact Mammalian Skeletal Muscle Fibers Expressing the Embryonic CaV1.1 Splice Variant

Beatrix Dienes[1], Nasreen Sultana[2], Janos Vincze[1], Monika Sztretye[1], Peter Szentesi[1], Bernhard E. Flucher[2], **Laszlo Csernoch**[1].
[1]University of Debrecen, Debrecen, Hungary, [2]Medical University of Innsbruck, Innsbruck, Austria.

The embryonic splice variant of the voltage-gated L-type calcium channel (CaV1.1e) displays an altered voltage-dependence and gating kinetics as compared to that expressed in adult skeletal muscle. Because the adult CaV1.1a only opens slowly at strong depolarizations, its contribution as a source of calcium influx during action potentials is negligible. In contrast, calcium influx through the embryonic CaV1.1e substantially contributes to depolarization-induced calcium transients in fetal muscles and in cultured myotubes. In a genetically modified mouse (CaV1.1αE29), which exclusively expresses the embryonic CaV1.1e variant also in adult muscle the calcium influx component is maintained throughout life. Utilizing this mouse model, calcium release events - calcium sparklets - were recorded in enzymatically isolated, intact adult skeletal muscle fibers from the m. flexor digitorum longus using the fluorescent calcium probe fluo-8 and the fast confocal scanner (ZeissLive) in the x-y mode. While control animals did not display such events, CaV1.1αE29 mice spontaneously generated sparklets with a frequency of 9.2*10-4 ± 6*10-4 Hz/μm2 (19 fibers; mean ± SEM). The role of external calcium as the trigger was tested by either removing calcium from the external solution or by the application of 5μM nizoldipine to block the calcium current through CaV1.1e. Both interventions resulted in a complete loss of the events. Identified sparklets (n=311) were characterized by an average amplitude (αF/F0) of 0.287 ± 0.005, a full-width at half-maximum of 3.05 ± 0.05 μm, and duration of 235 ± 4 ms, clearly different from the properties of calcium sparks on saponin-permeabilized adult mammalian skeletal muscle fibers. These findings indicate that the sustained expression of the CaV1.1e splice variant gives rise to spontaneous calcium entry events (sparklets) in adult muscle fibers and that their properties are distinct from calcium sparks arising from ryanodine receptors. Support: OTKA-NN107765, FWF P23479, W1101, TÁMOP-4.2.4.A./2-11-1-2012-0001.

2545-Plat

Calcium Channel Dysfunction in a Mutant Mouse Model of Malignant Hyperthermia(CaV1.1 R174W)

Donald Beqollari[1], Christin F. Romberg[1], Wei Feng[2], Jose R. Lopez[2], Manuela Lavorato[3], Stefano Perni[4], Philip M. Hopkins[5], Clara Franzini-Armstrong[3], Isaac N. Pessah[2], Paul D. Allen[2], Kurt G. Beam[4], **Roger A. Bannister**[1].
[1]Medicine-Cardiology, University of Colorado Denver-AMC, Aurora, CO, USA, [2]Molecular Biosciences, University of California-Davis, Davis, CA, USA, [3]Cell and Developmental Biology, University of Pennsylvania, Philadelphia, PA, USA, [4]Physiology and Biophysics, University of Colorado Denver-AMC, Aurora, CO, USA, [5]Leeds Institute of Biomedical and Clinical Sciences, University of Leeds, Leeds, United Kingdom.

Malignant hyperthermia (MH) is a potentially fatal pharmacogenetic disorder of skeletal muscle that is triggered by exposure to volatile anesthetics. MH has been studied extensively in mice and pigs carrying causative mutations in the type 1 ryanodine receptor (RyR1). However, no in vivo information exists regarding how mutations in the skeletal muscle L-type Ca^{2+} channel (CaV1.1) precipitate MH crises. For this reason, we generated a mouse line carrying the R174W mutation. Homozygous R174W mice ambulated efficiently, reproduced and had normal lifespans. When exposed to isoflurane, homozygous R174W mice entered a hypermetabolic state ending ultimately in death. On the ultrastructural level, R174W muscle displayed limited and variable changes: some variability of the SR calsequestrin content, displacement of mitochondria in some soleus fibers of aged mice and occasional accumulation of SR stacks. On the cellular level, homozygous R174W muscle had elevated resting myoplasmic Ca^{2+} levels that were greatly increased upon exposure to isoflurane. Flexor digitorum brevis (FDB) fibers dissociated from homozygous R174W mice lacked L-type Ca^{2+} current even though intramembrane charge movements of were of similar magnitude and voltage-dependence to those recorded from wild-type fibers. Ca^{2+} released from the SR in response to depolarization was substantially reduced in homozygous R174W fibers suggesting a depleted SR Ca^{2+} store. Lipid bilayer recordings showed that the Po of RyR1s isolated from homozygous R174W mice was significantly increased at all cis Ca^{2+} concentrations (Feng et al., this meeting). Taken together, our results support a mechanism for MH susceptibility in which CaV1.1 R174W promotes SR Ca^{2+} leak without affecting EC coupling per se. This work was supported by grants from the NIH (AR055104 to KGB, AR052534 to PDA, KGB, PMH, CFA and INP) and MDA (MDA277475 to KGB). DB received a stipend from 2T32AG000279-11.

2546-Plat

Spatially Localized Disruptions of Voltage Activated Calcium Release in Mtm1-Deficient Muscle Fibers

Candice Kutchukian[1], Karine Poulard[2], Anna Buj-Bello[2], **Vincent Jacquemond**[1].
[1]Centre for Molecular and Cellular Genetics and Physiology, UCB Lyon 1, UMR CNRS 5534, Villeurbanne, France, [2]Department of Research and Development, Généthon, INSERM, Evry, France.

Mutations in the gene encoding the phosphoinositide phosphatase myotubularin (*Mtm1*) are responsible for myotubular myopathy. We previously showed that muscle fibers from *Mtm1*-deficient mouse suffer from defective excitation-contraction coupling. Here we measured voltage-clamp activated Ca^{2+} signals in fibers from the flexor digitorum brevis muscles of 4 week-old wild type (WT) and *Mtm1*-ko mice under line-scan confocal microscopy using the dye rhod-2. Ca^{2+} release in the diseased fibers was deficient over the full range of activation: fitting the voltage dependence of the peak rate of rise of the line-averaged rhod-2 F/F_0 signals with a Boltzmann function gave mean values for maximum rate, midpoint voltage and slope of 0.29 ± 0.02 and 0.14 ± 0.02 $F/F_0.ms^{-1}$, −8.8 ± 1.5 and 0.95 ± 2.3 mV, 7.0 ± 0.4 and 9.2 ± 0.7 mV in WT (n=22) and *Mtm1*-ko fibers (n=15), respectively. Furthermore, the mean time to peak rate was significantly delayed by 5-10 ms in *Mtm1*-ko as compared to WT fibers. These global alterations were associated with severe spatial inhomogeneity of Ca^{2+} release in the diseased fibers with rhod-2 transients yielding localized disruptions along the scanned line including reduced peak amplitude but also delayed or slower rate of onset, suggestive of specific alteration of the early peak component of the rate of Ca^{2+} release. In fibers treated with wortmannin and LY294002, the properties of the line-averaged rhod-2 F/F_0 signals were unchanged in WT fibers but the mean maximum rate of rise of the rhod-2 signal was enhanced by 60% in *Mtm1*-deficient fibers. Results show that *Mtm1*-deficiency provokes EC coupling failure through accumulation of spatially localized disruptions of

Ca^{2+} release. They also suggest that pharmacological inhibition of PtdIns-3-kinase activity has the potency to alleviate defective EC coupling in the diseased fibers.

Platform: Mechanosensation

2547-Plat

The Primary Cilium is a Self-Adaptable, Integrating Nexus for Mechanical Stimuli and Cell Signaling

An M. Nguyen[1], Yuan N. Young[2], **Christopher R. Jacobs**[1].
[1]Biomedical Engineering, Columbia University, New York, NY, USA, [2]Mathematical Sciences, New Jersey Institute of Technology, Newark, NJ, USA.

Mechanosensation is critical for cells to maintain homeostasis and in devastating diseases, including atherosclerosis, osteoporosis and cancer. Although several cellular mechanosensing structures have been described, none have been shown to adapt to mechanical stimuli or be regulated in non-excitable cells. Primary cilia are ubiquitous chemo-mechanical sensors and function as mechanosensors in several tissues, including kidney, liver, cartilage, bone, and the embryonic node, deflecting in response to mechanical stimuli. Several groups have shown cilia length adapts in response to mechanical stimuli while others have shown relatively small changes in length can affect cilia deflection and downstream load-induced changes in gene and protein expression. Collectively, this suggests cilia mechanosensitivity may be modulated. Here, we show that both mechanical and chemical mechanisms can alter ciliary rigidity. We exposed mouse inner medullary collecting duct cells transfected with a live-cell marker for primary cilia to flow. Cilium bending behavior was captured with high-speed confocal microscopy and modeled as a beam anchored by a torsional spring. We found exposure to flow stiffened the cilium up to 4-fold (n=12), deflecting less in response to subsequent exposures to flow. We hypothesized that modifying tubulin may mimic this stiffening. Post-translational modifications of tubulin, such as acetylation, have been shown to stiffen microtubules. Interestingly, acetylation can increase with mechanical stimuli. Using a potent pharmaceutical agent and a siRNA knockdown to alter acetylation, we showed that through acetylation the cell can biochemically regulate ciliary stiffness up to 4-fold (n=5/group). We further showed that this altered stiffness directly affects the sensitivity of the cell to mechanical signals, resulting in a 2-fold change in gene expression (n=5/group). We demonstrated, for the first time, a potential mechanism through which the cell can regulate its mechanosensing apparatus.

2548-Plat

Mechanosensitive Von Willebrand Factor Protein-Protein Interactions Regulate Hemostasis

Camilo A. Aponte Santamaría[1], Volker Huck[2], Sandra Posch[3], Agnieszka K. Bronowska[1], Sandra Grässle[2], Maria A. Brehm[4], Tobias Obser[4], Reinhard Schneppenheim[4], Peter Hinterdorfer[3], Stefan W. Schneider[2], Carsten Baldauf[5], Frauke Gräter[1].
[1]Molecular Biomechanics, Heidelberg Institute for Theoretical Studies, Heidelberg, Germany, [2]Experimental Dermatology, Medical Faculty Mannheim, Heidelberg University, Mannheim, Mannheim, Germany, [3]Institute of Biophysics, Johannes Kepler University, Linz, Austria, [4]Pediatric Hematology and Oncology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany, [5]Theory, Fritz-Haber-Institut der Max-Planck-Gesellschaft, Berlin, Germany.

A recurring theme of mechanosensitive proteins is a cryptic binding site that gets uncovered by an external force. Here, we test the role of such mechanism in the adhesion of platelets at sites of vascular injury, a key process mediated by the von Willebrand factor (VWF). Our data from atomistic simulations, atomic force microscopy (AFM), and microfluidic experiments demonstrate that the VWF A2 domain binds to the VWF A1 domain, such that it buries the platelet binding site located at A1. This implies inactivation of VWF for the binding of platelets by a direct protein-protein interaction between the VWF A1 and A2 domains. During force-probe simulations and AFM experiments, a stretching force uncovered the platelet binding site, by dissociating the A1-A2 complex. This process was accompanied with only a partial unfolding of the A2 domain causing minor exposure of its cleavage site. Our data thus suggest that activation for platelet binding and degradation by cleavage are coupled through the interaction of A1 with A2, and force guarantees that VWF gets ready for activation before cleavage. Microfluidic experiments with an A2-deletion VWF mutant corroborate the critical inactivation role of the A2 domain *in vitro*. Overall, inactivation of VWF by force-dependent inter-domain A1-A2 interactions answers the question of how platelets are prevented to bind to VWF under equilibrium conditions. The notion of a cryptic protein binding site uncovered by force does not only provide the molecular basis for the long-standing observation of shear-dependent platelet binding during blood clotting, but it might also help to explain shear-induced VWF self-assembly.

2549-Plat

Structural Study of a Novel Partial Calcium-Free Linker and a Positively Selected Variation in Protocadherin-15: Implications for Hearing and Cell Adhesion

Robert E. Powers[1], Rachelle Gaudet[2], Marcos Sotomayor[3].
[1]Biophysics Graduate Program, Harvard University, Boston, MA, USA, [2]Department of Molecular and Cellular Biology, Harvard University, Cambridge, MA, USA, [3]Department of Chemistry and Biochemistry, The Ohio State University, Columbus, OH, USA.

Protocadherin-15 (PCDH15) is a non-classical cadherin that interacts with Cadherin-23 (CDH23) to form a heterophilic filament called the ''tip link''. The tip link is essential for hearing as it conveys sound-induced forces to the mechanosensitive ion channels responsible for the generation of electrical signals involved in sound perception. CDH23 and PCDH15 are part of the larger family of cadherin proteins that typically adhere cells together in a calcium-dependent manner and contain extracellular cadherin (EC) repeats. Recent research has identified an aspartate to alanine variation (D414A) within the linker region between EC3 and EC4 of PCDH15 that is under positive selection in East Asian populations. The EC3-4 linker region of PCDH15 also lacks several conserved calcium-binding residues, placing it in a recently identified class of cadherin linker regions that may be unable to bind Ca^{2+} ions at one or more of the canonical binding sites. To study the implications of the D414A variation and the noncanonical calcium-binding motifs, atomic resolution structures of the EC3-5D414 and the EC3-5A414 fragments of PCDH15 were determined by x-ray crystallography. The structures revealed a novel partial calcium-free linker region between EC3 and EC4 that only binds 2 Ca^{2+} ions. Preliminary molecular dynamics simulations revealed an increased flexibility for the EC3-4D414 linker region as compared to canonical cadherin linker regions that bind 3 Ca^{2+} ions. Furthermore, the simulations predict that the D414A variation may suppress this flexibility and rigidify the EC3-4 linker region, which could be advantageous given the role of PCDH15 in force transduction. The new structures represent a first step in determining the structural and dynamical properties of non-canonical EC repeats of PCDH15 with positively selected variations that are relevant for sensory perception.

2550-Plat

Calcium Influx through TRPV1 Inhibits Piezo Channels via Phosphoinositide Depletion

Istvan Borbiro, Doreen Badheka, Tibor Rohacs.
Dept. of Pharm. and Phys., Rutgers NJMS, Newark, NJ, USA.

Capsaicin, the activator of the noxious heat sensor TPRV1 is clinically used to relieve chronic pain. Desensitization of TRPV1 involves downstream PLCδ activation, but the mechanism how capsaicin treatment can also alleviate mechanical allodynia is unknown. Piezo2 encodes rapidly adapting mechanically activated currents in sensory neurons. Expression of these novel mechanosensitive ion channels in a subset of TPRV1 positive neurons suggests their role in pain related sensory mechanotransduction.

Our study shows that capsaicin application inhibited rapidly adapting mechanically activated currents in TRPV1 expressing sensory neurons. We also show that TRPV1 activation inhibited heterologously expressed Piezo1 and Piezo2 ion channels in whole-cell patch clamp experiments. Inclusion of either phosphatidylinositol 4,5-bisphosphate [$PI(4,5)P_2$] or its precursor PI(4)P in the patch pipette alleviated this inhibitory effect. Activation of PLCβ by stimulating muscarinic receptors only marginally inhibited mechanically activated Piezo1 currents. Experiments using phosphoinositide sensors revealed that activation of PLCδ by a robust calcium influx through TRPV1 severely depleted $PI(4,5)P_2$ and PI(4)P. On the other hand, muscarinic stimulation of PLCβ significantly decreased $PI(4,5)P_2$ levels, but only induced a small decrease of PI(4)P. This differential activation of PLC isoforms may explain the difference between the inhibitory effects of these PLC pathways. Targeted depletion of $PI(4,5)P_2$ and PI(4)P using a chemically inducible lipid phosphatase replicated the inhibition of Piezo1 currents. Additionally, $PI(4,5)P_2$ and PI(4)P applied to excised inside-out patches inhibited the rundown of Piezo1 activity further emphasizing the significance of these phosphoinositides in Piezo channel regulation.

Here we demonstrate that the activity of Piezo channels require the presence of either $PI(4,5)P_2$ or PI(4)P and severe depletion of both phosphoinositides by TRPV1 activation limits channel activity. In conclusion our data may explain how capsaicin alleviates mechanically induced pain.