levels of miR-1 and miR-133 were significantly increased in HF myocytes compared to controls (2 and 1.6 fold accordingly). Western blotting showed that PP2A regulatory (b56alpha) and catalytic subunits, specific targets of miR-1 and miR-133 validated by luciferase-reporter assay, were decreased in HF cells. Analysis using phospho-specific antibodies confirmed that RyR2 phosphorylation at Ser-2814 was significantly increased in HF myocytes compared to controls. CaMKII inhibitory peptide reduced the frequency of spontaneous Ca waves in paced current-clamped HF myocytes to low control values. These finding suggest that altered levels of major muscle-specific microRNAs contribute to abnormal RyR2 function in HF by depressing localized phosphatase activity to the channel, thus leading to excessive phosphorylation of RyR2s.

3045-Pos Board B150

Voltage-Dependent Anion Channel 2 modulates Resting Calcium Sparks, but not Action Potential-Induced Global Calcium Signaling in Cardiac Myocytes

Krishna P. Subedi, Joon-Chul Kim, Moonkyung Kang, Yeon-Soo Kim, **Sun-Hee Woo**.

Voltage-dependent anion channels (VDACs) are pore forming proteins predominantly found in the outer mitochondrial membrane and is thought to transport calcium ion (Ca^{2+}). In this study, we have investigated the possible role of type 2 VDAC (VDAC2) in cardiac Ca^{2+} signaling and Ca^{2+} sparks using a lentiviral knock-down (KD) technique and two-dimensional confocal Ca^{2+} imaging in immortalized autorhythmic adult atrial cells, HL-1. We confirmed high expression of VDAC2 protein in ventricular, atrial and HL-1 cells using Western blot analysis. Infection of HL-1 cells with VDAC2-targeting lentivirus reduced the level of VDAC2 protein to ~10%. Comparisons of autorhythmic Ca^{2+} transients between wild type (WT) and VDAC2 KD cells showed no significant change in the magnitude, decay, and beating rate of the Ca^{2+} transients. Caffeine (10 mM)-induced Ca^{2+} release, which indicates sarcoplasmic reticulum (SR) Ca^{2+} content, was not altered by VDAC2 KD. Interestingly, however, the intensity, width, and duration of the individual Ca^{2+} sparks were significantly increased by VDAC2 KD in resting conditions, with no change in the frequency of sparks. These results suggest that VDAC2 may suppress focal Ca^{2+} releases through ryanodine receptors in atrial myocytes under resting conditions. The results also indicate that VDAC2 may not regulate action potential-induced global Ca^{2+} signaling and SR Ca^{2+} loading.

3046-Pos Board B151

African Trypanosomes Increase Calcium Wave Frequency in Isolated Adult Rat Cardiomyocytes via Secretion of Cathepsin L

Elspeth B. Elliott, Liam J. Morrison, Hisashi Hasumi, Christopher M. Loughrey.

African trypanosomes are blood-borne extracellular parasites which have recently been linked to cardiac dysfunction in ~70% of sleeping sickness patients. Although this may result from an indirect effect of the parasite (e.g. myocarditis), a direct effect of the parasite on the heart has not been investigated. Adult rat cardiomyocytes were incubated with trypanosome growth media containing *Trypanosoma brucei* Lister427 (30min). A population assay assessed the percentage of cells demonstrating Ca^{2+} waves within a 1min period. Incubation with live trypanosomes led to a significant increase in the percentage of cells demonstrating Ca^{2+} waves ($54.8 \pm 2.8\%$ *vs.* $79.2 \pm 5.1\%$; media *vs.* live trypanosomes, $P<0.05$; n=4294 and 3006 cells respectively). This effect was maintained when cells were incubated with supernatant (trypanosomes removed from media by centrifugation) ($77.3 \pm 2.9\%$; n=2131 cells). Separate experiments showed the supernatant effect was lost upon boiling ($83.7 \pm 1.8\%$ *vs.* $66.3 \pm 2.4\%$; supernatant *vs.* boiled supernatant, $P<0.05$; n=527 and 612 cells respectively). Results were confirmed in Fura2AM loaded, field stimulated (1Hz) rat cardiomyocytes perfused with media (37°C). Following 4 min supernatant perfusion, the frequency of Ca^{2+} waves in the inter-stimuli interval was significantly increased (0.02 ± 0.01 *vs.* 0.44 ± 0.07 waves/s; media *vs.* supernatant, $P<0.05$; n=10). Since the parasite induces a similar phenomenon in brain mono-epithelial cells via cathepsin-L cysteine protease, we examined the role of cathepsin-L in the above effect on cardiomyocytes. In separate experiments, supernatant+K11777 (specific inhibitor of cathepsin-L) completely abolished the ability of supernatant to increase Ca^{2+} wave probability (56.3 ± 5.1 *vs.* $49.1 \pm 5.7\%$; media *vs.* supernatant+K11777, $P>0.05$), whereas CA074 (specific inhibitor of cathepsin-B) had no effect on Ca^{2+} wave frequency. These data suggest trypanosomes interact with cardiomyocytes leading to increased Ca^{2+} wave production via cathepsin-L. This may contribute to the cardiac abnormalities observed in patients with trypanosomiasis.

3047-Pos Board B152

Calcium Handling in Human Induced Pluripotent Stem Cell-Derived Cardiomyocytes

E. Michelle Capes, Randall E. Loaiza, Roberto Ramos, Jianhua Zhang, Timothy J. Kamp, Hector H. Valdivia.

Fibroblasts from human skin biopsies can be reprogrammed into pluripotent stem cells (iPSC), which can then be coaxed to differentiate into myocytes with cardiac-specific properties (iPSC-CMs). The field of iPSCs is still in its infancy, but it is increasingly clear that the excitation-contraction coupling (ECC) machinery of differentiating CMs undergoes proportionally incremental complexity and it remains to be seen whether it reaches complete maturity in cultured cells. We used patch-clamp and confocal Ca^{2+} imaging for a comparative assessment of ECC in human iPSC-CM and adult cardiomyocytes. In the latter, entry of Ca^{2+} through the L-type Ca^{2+} channel (I_{Ca}) triggers rapid, uniform release of Ca^{2+} from the sarcoplasmic reticulum (SR) via CICR. In iPSC-CMs at early stages of differentiation, the current-voltage relationship for I_{Ca} is remarkably similar to that of adult cardiomyocytes, indicating that the appearance of a "trigger" for contraction is an early event in the ontogenesis of ECC that doesn't hinder efficient generation of Ca^{2+} signals. However, primitive iPSC-CMs commonly exhibit a poorly developed SR, as assessed by their variegated response to caffeine and their great dependence on extracellular Ca^{2+} for contraction. Cells are mostly rounded and t-tubules are absent. As a result, $[Ca^{2+}]_i$ transient waveforms appear non-uniform and start at the periphery of the cell, as is expected of a Ca^{2+} front with focal initiation that propagates later to the interior of the cell. At more advanced stages of differentiation, iPSC-CMs display fairly uniform Ca^{2+} fronts, suggesting fast propagation of external Ca^{2+} signals to the interior of the cell. Thus, by this coarse functional estimate, it is expected that iPSC-CMs become accurate models of cardiomyopathies at late stages of differentiation, but the developmental characteristics of ECC is unclear and warrants a systematic approach, which we are currently performing.

3048-Pos Board B153

Impaired Calcium Signaling Refractoriness Contibutes to Increased Rate of Diastolic Calcium Waves in Myocytes from Post-Myocardial Infarction Hearts

Andriy E. Belevych, Dmitry Terentyev, Radmila Terentyeva, Hsiang-Ting Ho, Cynhtia A. Carnes, George E. Billman, Sandor Gyorke.

Spontaneous Ca^{2+} waves (SCWs) are recognized as important contributors to triggered arrhythmia. SCWs waves are thought to arise when $[Ca^{2+}]_{SR}$ reaches a certain threshold level, which might be reduced in cardiac disease as a consequence of sensitization of ryanodine receptors (RyR2s) to luminal Ca^{2+}. We investigated the mechanisms of SCW generation by simultaneous measurements of cytosolic and luminal Ca^{2+} in myocytes from normal and diseased hearts using a canine model of post-myocardial infarction (MI) tachyarrhythmia. The frequency of SCW, recorded during periodic pacing in the presence of β-adrenergic receptor agonist isoproterenol, was significantly higher in MI myocytes than in control. Rather than occurring at once upon reaching a final $[Ca^{2+}]_{SR}$, SCWs arose with a distinct time delay from the attainment of the maximum $[Ca^{2+}]_{SR}$ in both experimental groups. While the rate of $[Ca^{2+}]_{SR}$ recovery following the SR Ca^{2+} release was similar between the two myocyte types, the maximally attainable $[Ca^{2+}]_{SR}$ was lower, and the latency to SCW was shorter in MI myocytes compared to control. Both phosphorylation at the CAMKII site Ser-2814 and the level of oxidized thiols were higher in RyR2s from MI hearts than in control. The CAMKII inhibitor, KN93, or the reducing agent, mercaptopropionylglycine, reduced SCW frequency in MI myocytes. The MI-related alterations in myocyte Ca^{2+} cycling were mimicked by the RyR2 agonist, caffeine. These results indicate that attainment of a certain threshold $[Ca^{2+}]_{SR}$ is not a sufficient condition for the generation of SCWs and that Ca^{2+} signaling refractoriness that develops following release critically influences SCW occurrence in the diastolic period. We conclude that shortened Ca^{2+} signaling refractoriness due to RyR2s phosphorylation and oxidation is responsible for the increased rate of SCWs observed in MI myocytes.

3049-Pos Board B154

Inositol 1,4,5 Triphosphate (IP3) Receptors Activate Type 1 ryanodine Receptors to Mediate Ca^{2+} Sparks Signaling in Adult Mammalian Skeletal Muscle

Andoria Tjondrokoesoemo, Na Li, Noah Weisleder, Jianjie Ma.

Ca^{2+} sparks are the elemental event of Ca^{2+} induced Ca^{2+} release (CICR) that originate from clustered ryanodine receptor Ca^{2+} release channels (RyR1) in mammalian striated muscles. Previously we found that application of transient osmotic stress to the intact skeletal muscle leads to a robust Ca^{2+} spark response that is restricted to the periphery of sarcolemmal membrane. Here we

determine the fundamental mechanism for CICR activation in mammalian skeletal muscle. Transient osmotic stress increases PI(4,5)P2 and inositol 1,4,5 triphosphate (IP3) levels. Application of wortmannin or xestopongin C significantly reduces osmotic stress-induced Ca^{2+} spark activity in intact muscle fibers, suggesting the role of IP3 receptor in Ca^{2+} spark signaling. Western blot shows that both IP3 receptor type 1 and 2 are present in adult skeletal muscle, and immunostaining reveals that both IP3 receptors are distributed along the sub-sarcolemmal region of the muscle fiber (with some concentrated to the perinuclear area). Using electroporation mediated transfection to deliver short hairpin (sh)RNA that targets IP3 receptors, we are able to knockdown the expression of both IP3 receptors 1 and 2 in the muscle of viable adult mice. We find that reduced expression of IP3 receptors ablates osmotic stress-induced Ca^{2+} spark activity, indicating Ca^{2+} sparks activity in skeletal muscle requires activation of IP3 receptor. Thus, osmotic stress-induced Ca^{2+} spark signaling in skeletal muscle requires two cellular events: first, uncoupling of the inhibitory role of the voltage sensor on the RyR1 channels, and second, production of the IP3 second messenger near the sarcolemmal membrane. These results represent the first description of IP3 receptors producing CICR from RyR1 in mammalian skeletal muscle and provide essential clues to the function of these Ca^{2+} sparks in skeletal muscle physiology.

3050-Pos Board B155

Recovery of the Compromised Ca^{2+} Spark Signaling in Aged Skeletal Muscle Through Restoration of MG29

Xiaoli Zhao, Norio Takizawa, KiHo Park, Kyoung-Han Choi,
Hilary A. Wilkinson, Dennis M. Zaller, Noah Weisleder, Jianjie Ma.

Sarcopenia is a degenerative loss of skeletal muscle function associated with aging. Our previous results identify reduced MG29 expression in aged skeletal muscle, and mirroring phenotypes of the young MG29 knockout and aged wild type muscles in that both show reduced Ca^{2+} spark response to osmotic-stress. Thus, compromised intracellular Ca^{2+} homeostasis due to reduced MG29 expression may be one of the underling mechanisms for aging-related skeletal muscle dysfunction. Here we explored the effects of MG29 rescue on Ca^{2+} spark signaling in aged skeletal muscle. Electroporation-based method was used to introduce MG29 into *flexor digitorum brevis* (FDB) muscle and adeno-associated virus (AAV)-based method was used to deliver MG29 gene into the hindlimb of the living mice. Confocal microscopic imaging revealed increased Ca^{2+} spark events in aged FDB muscle following transient overexpression of MG29. These Ca^{2+} sparks showed plastic response to osmotic stresses, similar to those observed in the young wild type muscle. 2-3 weeks following AAV-mediated delivery of MG29, the aged skeletal muscle showed only marginal increase in contractile force as compared to the contralateral controls. Our data suggest that transient restoration of MG29 expression in aged muscle has beneficial effects on improvement of intracellular Ca^{2+} signaling. Since MG29 is involved in maintenance of the transverse-tubule network, restoration of contractile force in aged muscle may require sustained elevation of MG29 to allow for remodeling of the disrupted membrane network.

3051-Pos Board B156

Hypersensitive Intracellular Ca^{2+} Signaling Precedes Deterioration of Cardiac Functions in Muscular Dystrophy

Sergii Kyrychenko, Eva Poláková, Krisztina Poscai, Nina D. Ullrich,
Ernst Niggli, Natalia Shirokova.

Duchenne muscular dystrophy (DMD) is a severe form of striated muscle disease. Although respiratory failure remains a leading cause of death, a number of patients succumbs from cardiac manifestations of the disease. The *mdx* mouse, an animal model of DMD, develops progressive dilated cardiomyopathy. Several studies associated changes in Ca^{2+} homeostasis with the disease. Here we investigated whether these changes were causal for or a consequence of the pathology. Ca^{2+} handling was studied in intact and patch-clamped cardiomyocytes isolated from 1 to 4 month old mice. According to several reports, young *mdx* mice show no significant changes in cardiac performance. However, even myocytes from 1 month old *mdx* mice produced exaggerated Ca^{2+} signals in response to osmotic shock, and exhibited ''hypersensitive'' excitation-contraction coupling (ECC gain was more resistant to a reduction in $[Ca^{2+}]_{ex}$ in *mdx* than in WT cells). Ca^{2+} transients induced by osmotic shock were nearly abolished by the super-oxide dismutase mimetic Mn-cpx3, substantially reduced by a CaMKII inhibitor (KN-93) and partially diminished by PKA inhibitors (KT5720, H89). No significant changes in SR Ca^{2+} load as well as in resting $[Ca^{2+}]_i$ were found in young *mdx* compared to WT cells. Together with our previous results, these data suggest that 1) increased sensitivity of RyRs to Ca^{2+} precedes and probably contributes to the development of cardiomyopathy in dystrophy and that 2) there is a synergistic interaction among several pathomechanisms which hypersensitize the RyR. This includes a) abnormal Ca^{2+} influx resulting in b) cellular Ca^{2+} overload, c) elevated ROS generation leading to RyR redox modification and sensitization, and d) activation of protein kinases with subsequent RyR phosphorylation and even further sensitization. Thus, future pharmacological strategies should preferably target several of these mechanisms contributing to abnormal Ca^{2+} signals in DMD.

3052-Pos Board B157

A Novel Role for Polyphosphate in Astrocyte Signalling

Kira M. Holmstrom, Alexander V. Gourine, Andrey Y. Abramov.

Inorganic polyphosphate exists in nature in varying lengths from tens to thousands of orthophosphates linked by high energy bonds similar to ATP. The polymer is highly conserved from bacteria to human, but although its role has been extensively studied in bacteria, its function in the mammalian cell is only slowly coming to light. Polyphosphate has been detected in the rodent brain at micromolar concentrations and has been shown to regulate ion channels in neurons, suggesting that polyphosphate may play a role in neuronal signalling.

We used fluorescent live cell imaging to investigate the response to polyphosphate in primary astrocytic and neuronal co-cultures. For the experiments three different lengths of polyphosphate (short −14, medium −60, and long −130, orthophosphates) were used. Further, using the ratiometric Ca^{2+} indicator fura-2, we were able to identify a transient Ca^{2+} signal, mainly in astrocytes, in response to polyphosphate in the range of 10-100μM for all three lengths of the polymer. Interestingly, inhibiting phospholipase C by U73122 abolished the Ca^{2+} transient, as did emptying the endoplasmic reticulum of Ca^{2+} before addition of polyphosphate, using the sarco/endoplasmic reticulum Ca^{2+} ATPase inhibitor thapsigargin. On the other hand, removal of Ca^{2+} from the extracellular recording medium did not alter the signal, suggesting that the Ca^{2+} signal stems from the endoplasmic reticulum and is mediated through phospholipase C and IP_3 activation. Further characterisation, using different cell surface receptor inhibitors, suggests that the signal is mediated through purinergic receptors, as the broad spectrum P2 inhibitors PPADS and suramin both block the signal.

These novel findings highlight the possible importance of polyphosphate in signal transmission in the brain.

3053-Pos Board B158

Dynamic Control of Neuronal Firing Threshold by Calcium Buffering: A New Role for Calcium Binding Proteins

Patrick Bischop, Céline Roussel, David Orduz, Serge N. Schiffmann,
David Gall.

We have investigated the detailed regulation of neuronal firing threshold by the cytosolic calcium buffering capacity using a combination of mathematical modeling and patch clamp recording in acute slice. Theoretical results show that, at similar free calcium concentration, increased calcium buffer concentration lowers the firing threshold of cerebellar granule cells. We show that this effect is a direct consequence of the major slowdown of calcium dynamics. Patch clamp recordings on cerebellar granule cells loaded with a high concentration of the fast calcium buffer BAPTA (15 mM) reveal alterations in the excitability threshold as compared to cells loaded with 0.15 mM BAPTA. In high calcium buffering conditions, granule cells exhibit a significative lower firing threshold. These results suggest that cytosolic calcium buffering capacity can tightly modulate neuronal firing threshold and therefore that calcium-binding proteins may play a critical role in the information processing in the central nervous system.

Intercellular Communications & Gap Junctions

3054-Pos Board B159

Single Hemichannels Recorded in Lipid Bilayers and Artificial Gap Junction Formation with Cells

Mohamed Kreir, Christoph Methfessel, Christian Carnarius,
Claudia Steinem, Niels Fertig.

Connexins (Cx) are members of a multigene family of membrane-spanning proteins that form gap junctions, which are composed of two hexameric hemichannels, called connexons. These gap junctions, organized in so-called gap junctional plaques, span the extracellular space/matrix of adjacent cells and thus allow a passive exchange of small molecules up to about 1 kDa. Connexins are widely distributed with various subtypes of connexin and are involved in different biological processes such transmission of information and propagation of action potential for e.g. Recent studies indicates that hemichannels do open under physiological and pathological conditions.