increases evoked in response to IP_3, released locally in two parts of the cell, did not annihilate but approximately doubledin amplitude. This result suggests that the $[Ca^{2+}]_c$ increase, generated by local release of IP_3, had not regeneratively propagated but diffused passively from the release site. Notwithstanding, Ca^{2+} was required for IP_3-mediated wave progression to occur. Increasing the Ca^{2+} buffer capacity in a small (2 μm) restricted region of the cell immediately in front of a carbachol-evoked Ca^{2+} wave, by photolyzing the caged Ca^{2+} buffer diazo-2, halted progression at the site of photolysis. Failure of local increases in IP_3 to evoke waves appears to arise from the restricted nature of the IP_3 increase to small areas within the cell. When IP_3 was elevated throughout a localized increase in Ca^{2+} propagated as a wave. Together, these results suggest that waves initiate over a relatively large length of the cell and both IP_3 and Ca^{2+} are required for active propagation of the wave-front to occur.

1534-Pos

Increased Calcium Response to Depolarization in Voltage Clamped Skeletal Muscle Cells of a Transgenic Model of Amyotrophic Lateral Sclerosis

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Mitochondrial Ca uptake is believed to help regulate mitochondrial metabolism and synthesis of ATP to meet the demands of muscle contraction. Whether mitochondrial Ca uptake modifies Ca signaling during EC-coupling remains an open question. While studies show that mitochondria in skeletal muscle may take up Ca during contraction, it is not known whether altered mitochondrial Ca uptake can play a role in pathophysiological conditions. Our study on ALS mouse model G93A shows that ALS muscle fibers display defective mitochondria with loss of their inner membrane potential in fiber segments. The finding of localized mitochondrial defects in ALS fibers presents a unique opportunity to test whether changes in mitochondrial function can affect intracellular Ca signaling, as Ca release activity can be compared in regions with or without depolarized mitochondria in the same muscle fiber. By loading muscle fibers with TMRE (a probe of mitochondrial membrane potential) and fluo-4 (a Ca indicator) we characterized simultaneously mitochondrial function and Ca release activity in living muscle fibers. The fiber segment with depolarized mitochondria shows greater osmotic stress-induced Ca release activity. Abolishing mitochondrial inner membrane potential by FCCP or blocking mitochondrial uniporter by Ru360 exacerbates the osmotic stress-induced hyperactive Ca release. Furthermore, we evaluated the voltage-induced Ca transient by patch-clamping ALS fibers and found that fiber segments with depolarized mitochondria displayed 5~25% greater Ca transients. Our study constitutes a direct demonstration of the importance of mitochondria in shaping cytosolic Ca signaling in skeletal muscle. Malfunction of mitochondrial Ca uptake may play an important role in muscle degeneration of ALS. Supported by MDA/NIH.

1535-Pos

CICR and Calcium-Dependent Inactivation, Quantified Through the Response to Artificial Ca Sparks in Single Muscle Cells

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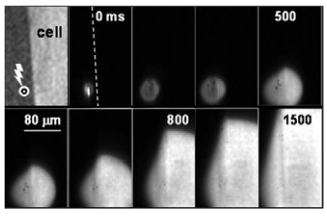
Local calcium stimuli (artificial sparks) generated by 2-photon breakdown of the cage NDBF-EGTA were applied to evoke Ca release from the SR in single skeletal or cardiac muscle cells undergoing fast Ca imaging with the low affinity dye fluo 4FF. The figure shows selected sequential images of the Ca transient generated by a frog skeletal muscle fiber with permeabilized plasmalemma, in response to a spark (elicited *outside* the fiber to avoid photodamage). Two types of responses were observed: (i) an all-or-none wave -shown- that propagates over the entire cell and (ii) graded responses, which fail to propagate. Release analysis (Ríos, JGP 1999; Figueroa, this meeting) separates SR release from simple diffusion of photo-released Ca into cells. The technique yields a sensitive measure of threshold $[Ca^{2+}]$ for release activation, which in the example (0.3 mM $[Mg^{2+}]_{cyto}$) was 1 μM, and can monitor inactivation by combining multiple stimuli. Modeling of these responses aims at describing quantitatively the properties of activation, as well as the roles of inactivation and depletion in the control of Ca release. Other details and acknowledgments are presented elsewhere (Figueroa, this meeting.)



1536-Pos

Flux in Artificial Ca Sparks Generated by 2-Photon Release from a Novel Cage Confocally Imaged at Microsecond Resolution

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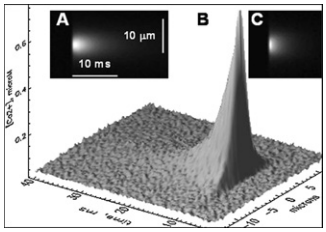
Control of calcium signaling in striated muscle relies on concurrent actions of calcium ions to promote and inhibit release channel opening. To understand these actions we developed artificial Ca sparks generated by 2-photon (2P) release from NDBF-EGTA (Momotake, Nature Methods 2006) as quantifiable local stimuli. A ''Dual Scanner'' (Zeiss) delivers IR laser flashes through a LSM 510 scanner, while rapidly imaging fluorescence of a $[Ca^{2+}]$ monitor via a slit scanner (5-LIVE; *ca* 100 μs/line). Ca sparks of 0.1 to 10 μM (A, B) are elicited in a droplet after microseconds of 2P irradiation at 720 nm and imaged with the low affinity dye fluo 4FF. Reaction-diffusion analysis (Ríos, JGP 1999) yields the flux of Ca photorelease (C). This flux, which initially reaches several hundred mM/s, decays with τ of 2-3 ms. The technique is used to measure physico-chemical properties of calcium ligands, including bio-sensors. Applied inside muscle fibers (Figueroa, this meeting) it serves to quantitatively characterize calcium control in cells.



Instrument purchased with a S10 NCRR award and Hasterlik Family matching funds. Supported by NIAMS, NHLBI/NIH and MDA.

1537-Pos

Effects of High [BAPTA] Inside Mouse Muscle Fibers Reveal a Role of Calcium in the Termination of Voltage-Operated Calcium Release from the SR

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Striated muscles have a termination mechanism that causes the flux of Ca release from the SR, whether activated by an action potential or a voltage pulse, to rapidly cease after an early peak. This mechanism is viewed as a fundamental property that insures stability and ''gradedness'' in the control of Ca signals. To probe this termination, the evolution of Ca release flux was derived from cytosolic Ca transients elicited by SR-depleting depolarizations of long duration, in voltage-clamped cells of mouse FDB muscle. In the presence of 5 mM of the Ca buffer BAPTA, the release flux underwent major changes compared with a 10 mM [EGTA] ''reference'' situation (studied by Royer, J Physiol 2008). Ca release reached an early peak and then decayed to a sustained phase that was higher and briefer than in reference, often including a second rise or ''hump''. In BAPTA, measurable release only lasted 100 ms or less. Its time integral -which measures the SR Ca content releasable by depolarization_was on average 1.4 mM (n=12 cells), compared with 2.1 mM for 18 cells in reference.

An increase in flux with conserved releasable content indicates that BAPTA promotes flux and hastens emptying of the SR without greatly changing its storage properties (including lumenal $[Ca^{2+}]$). A greater Ca flux driven by a similar $[Ca^{2+}]$ gradient requires greater and/or more sustained channel openness. These observations suggest the presence of a release channel-inhibiting mechanism (CDI) mediated by binding of cytosolic Ca^{2+} to open or closed channels, a mechanism more susceptible to interference by BAPTA than the slower-reacting EGTA. Work funded by NIAMS/NIH and an MDA grant to Dr. Jingsong Zhou, who we thank for continued support.

1538-Pos

D4cpv-Casq1. A Novel Approach for Targeting Biosensors Yields Detailed Dynamic Imaging of Calcium Concentration Inside the Sarcoplasmic Reticulum of Living Cells

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Dynamic imaging of $[Ca^{2+}]$ inside the SR of skeletal muscle is hampered by the limited sensitivity of available ratiometric biosensors (Rudolf, JCB 2006) and faces difficulties of calibration when using non-ratiometric dyes (Kabbara and Allen, J Physiol 2001). Impressed by the apparently perfect targeting of

calsequestrin 1 (Casq1) and its fusion constructs to terminal cisternae of the SR, we deviced an alternative approach: the fusion of murine Casq1 and D4cpv -a cameleon of high dynamic range and affinity adequate for the SR (Palmer, Chem & Bio 2006) - was expressed in the FDB of live adult mice. The fusion protein expressed well, localizing largely to terminal cisternae. Calibrations in situ revealed a good dynamic range of its Ca^{2+}-dependent ratio signal ($R_{max}/R_{min} = 4$). Sensor affinity is being measured using untargeted sensor, expressed in the cytosol, or purified sensor in solution. Assuming a $K_d = 240$ μM, the resting $[Ca^{2+}]_{SR}$ was 0.6-1 mM in 20 cells. Consistent with the kinetics of the related sensor D1 in solution ($kon = 256\ s^{-1}$, Palmer, PNAS 2004), D4cpv signals rapidly followed the decrease in $[Ca^{2+}]_{SR}$ that results from Ca^{2+} release upon voltage-clamp depolarization. Potential interference by the presence of Casq in the fused sensor was minimized by using a deletion mutant of Casq1 as targeting sequence. A first conclusion is that long-lasting depolarization may reduce $[Ca^{2+}]_{SR}$ below 10% of resting value. From the Casq1-D4cpv-monitored $[Ca^{2+}]_{SR}$ we derived the SR Ca buffering power -ratio of total/free $[Ca^{2+}]_{SR}$- and found that it decreases upon depletion of SR Ca. As shown elsewhere (Sztretye et al, this meeting), this anomalous buffering feature depends on the presence of calsequestrin inside the SR. Funded by NIAMS/NIH and MDA.

1539-Pos

Ca Depletion and Ablation of Calsequestrin Similarly Increase the Evacuability of the Ca Store of Skeletal Muscle

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At ~200 ms during a voltage pulse the flux of Ca release induced by membrane depolarization of mouse muscle exhibits a characteristic acceleration of decay, or shoulder, associated with SR depletion. The shoulder reflects an increase in evacuability *E*, an index calculated from the flux, equal to the ratio of release permeability, *P*, and SR Ca buffering power, *B* (Royer, J Physiol 2008). To tell whether this rise in *E* reflects an increase in *P* or a decrease in *B* we recorded flux and calculated *E* in FDB cells from mice lacking either calsequestrin 1 (Paolini, J Physiol 2007) or both isoforms of calsequestrin, the main Ca buffer in the SR. In both null mice the flux waveform lacked the shoulder, and *E* was elevated, adopting from the start the high value reached upon depletion in the wild-type. Hence, low *E* requires the presence of calsequestrin inside the store. In aqueous solutions the Ca-binding capacity of calsequestrin decreases at low $[Ca^{2+}]$ (Park, JBC 2005). Therefore we hypothesized that the increase in *E* during a pulse is due to an analogous effect of decaying $[Ca^{2+}]_{SR}$ on the Ca-buffering capacity of calsequestrin *in situ*. Confirming the hypothesis, when the SR was depleted in cells voltage-clamped in zero Ca external solutions, the kinetics of release became similar to that of calsequestrin-null cells, featuring no shoulder and a high initial *E*.

Low evacuability simply implies that the SR may release Ca with minimal decrease in $[Ca^{2+}]_{SR}$, therefore conserving the driving force for subsequent release. A functional correlate is the ability to sustain Ca release and Ca transients during the high frequency activation of physiological contractions and exercise. Funded by NIAMS/NIH and MDA.

1540-Pos

Compromised Ca^{2+} Sparks Signaling in the Skeletal Muscle of Diabetic Type 2 Mice

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Type 2 diabetes mellitus (DM) is a prevailing epidemic metabolic disease that is mainly characterized as insulin resistant and β-cell dysfunction that leads to aberrant glucose metabolism in skeletal muscle. Altered homeostatic capacity for effective $[(Ca^{2+})_i]$ signaling may underlie the reduced contractile dysfunction associated with DM. Measurement of osmotic stress induced Ca^{2+} sparks on the young control wild-type (WT)C57Bl/6J and db/db type 2 DM mice models show that Ca^{2+} sparks frequency is significantly attenuated in the db/db fibers (36 ± 6 events/min) when compared to control (107 ± 7 events/min). These findings suggest that Ca^{2+} sparks can be used as a readout of the Ca^{2+} handing characteristic of skeletal muscle fibers, as we have previously shown in muscular dystrophy and aging muscle. This idea is supported with additional studies that show therapeutic agents for diabetes can modulate Ca^{2+} spark signaling. Treatment of 10 nM glucagon like peptide 1 (GLP1), an incretin hormone associated with increased insulin secretion, significantly increases the Ca^{2+} sparks frequency in the db/db muscle (120 ± 10 events /min), similar to the level of untreated WT. The plot of Ca^{2+} sparks localization shows that treatment of GLP1 in either db/db or WT does not alter the peripheral subsarcolemmal distribution of Ca^{2+} sparks, implying that the factor responsible for maintaining Ca^{2+} sparks localization near the membrane remains intact. Studies in β-cell suggests that increased specific intracellular signaling cascade can be regulated by GLP-1. We find that these signaling cascades can also contribute to the activation of Ca^{2+} sparks in skeletal muscle.

Local Calcium Signaling

1541-Pos

A Technique to Accelerate Stochastic Markov Chain Monte Carlo Simulations of Calcium-Induced Calcium Release in Cardiac Myocytes

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Considerable insight into intracellular calcium (Ca) responses has been obtained through the development of whole cell models that are based on molecular mechanisms, e.g., the kinetics of intracellular Ca channels and the feedback of Ca upon these channels. However, a limitation of most deterministic whole cell models to date is the assumption that channels are globally coupled by a ''common pool'' of [Ca], when in fact channels experience localized ''domain'' [Ca]. More realistic stochastic Monte Carlo simulations are capable of capturing the influence of local [Ca] on channel gating. Unfortunately, such local control models of calcium-induced calcium release (CICR) are computationally expensive due to the explicit representation of 10,000 to 20,000 release sites, each containing 50 to 300 stochastically gating Ca channels. Here, we present a novel technique called vectorized gating that optimizes the solution time of Markov chain Monte Carlo (MCMC) simulations. Additionally, as this technique leverages vector and matrix algebra it can benefit from the use of the advanced NVIDIA TESLA graphics processing unit (GPU) to further accelerate MCMC models. NVIDIA TESLA cards utilize the parallel nature of the NVIDIA CUDA architecture and are powered by up to 960 parallel processing cores. Benchmark simulations indicate that the GPU-enhanced vectorized gating technique is significantly faster than CPU-only driven calculations. The vectorized gating technique also lends itself to the direct calculation of an adaptive time step which optimizes speed while maintaining numerical stability. These computational enhancements are utilized to facilitate study of sarcoplasmic reticulum (SR) leak from clusters of ryanodine receptor (RyR) Ca channels in cardiac myocytes.

1542-Pos

In Situ Calibration of Cytoplasmic and Nucleoplasmic Calcium Concentration in Adult Rat and Mouse Cardiac Myocytes

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Quantifying subcellularly resolved Ca^{2+} signals in cardiac myocytes is essential for understanding Ca^{2+} fluxes in excitation-contraction and excitation-transcription coupling. Translation of changes in Ca^{2+}-dependent fluorescence into changes in $[Ca^{2+}]$ relies on the indicator's behavior *in situ,* but properties of fluorescent indicators in different intracellular compartments may differ. Thus, we determined the *in situ* calibration of a frequently used Ca^{2+} indicator, Fluo-4, and evaluated its use in reporting cytoplasmic and nucleoplasmic Ca^{2+} signals in isolated cardiac myocytes.

Calibration solutions were made by mixing known quantities of EGTA and CaEGTA solutions and the free $[Ca^{2+}]$ was confirmed with a Ca^{2+}-sensitive electrode. Solutions contained metabolic inhibitors and cyclopiazonic-acid (5μM) to block active Ca^{2+} transport and the Ca^{2+} ionophore A-23187 (10μM) to allow equilibration of $[Ca^{2+}]$ between bath solution and cell interior. Ventricular rat and mouse myocytes were loaded with Fluo-4/AM (8μM, 20min). Fluo-4 fluorescence (excitation/emission: 488/>505nm) was recorded using a Nipkow dual disc-based confocal microscope.

Concentration-response curves were obtained and a significant difference in the apparent Ca^{2+} binding affinities (K_d) of Fluo-4 between cytoplasmic (993 ± 56nM; 1026 ± 65nM) and nucleoplasmic (1211 ± 73nM; 1251 ± 71nM) compartments was observed for both mouse and rat cells, respectively (both $n=15$, $P<0.01$). The established curves were used to transform raw Fluo-4 fluorescence signals during electrically stimulated $[Ca^{2+}]$ transients