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

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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