release flux was observed 3.9 ms after initiation of Ca release when all release sites became active. Ca entry flux was ten times smaller than Ca release flux. When SR Ca release was eliminated after treatment with thapsigargin and caffeine, identical Ca entry flux was observed as in control conditions. In summary, ultra-fast confocal imaging allows investigation of Ca signals with time resolution similar to patch clamp technique, however in a much less invasive fashion.

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Automatic Calcium Spark Detection and Analysis in Time Series of Two-Dimensional Confocal Images

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Calcium sparks are highly localized, brief calcium transients reflecting elementary calcium release from the sarcoplasmic reticulum that can occur either spontaneously or can be triggered during excitation contraction coupling in cardiac myocytes. They hold physiological as well as pathophysiolgical importance. Classically, calcium sparks were almost exclusively analyzed in line scan images because of technical limitations in the acquisition process consequently neglected their 2D spatial properties and distribution inside the cell.

During recent years the increased performance of confocal microscopes enabled high spatial and temporal resolution imaging. This allowed recording of calcium sparks from individual myocytes in time series of two-dimensional confocal images at acquisition rates exceeding 200Hz.

Here, we introduce an automatic three-dimensional approach for such analysis. Following cell border recognition we utilised locally ''derivative-like'' functions for spark-detection allowing the algorithm to analyse the temporal and spatial properties of calcium sparks. Such an approach revealed a highly robust spark-detection process, even when partial or global calcium waves occurred intermittently. 2D-gaussian fits over time were used to quantify such properties including amplitudes, decay time, frequency, spatial position and spread for further analysis. In addition, the algorithm automatically performs cluster analysis of the sparks found in order to identify common spark sites.

In an initial proof-of-concept study, we analysed spontaneous calcium sparks from various origins within the mouse heart (ventricle, left/right aria). The major result of this study was that the sparks display very distinct and characteristic properties that can be linked to physiology of the particular host cell.

Such a novel approach enables us to automatically analyze large populations of local calcium signals and thus fosters the use of such algorithms for novel high-content screening applications.

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1417-Pos Board B261

Image-Based Monte Carlo Modeling of Ca^{2+} sparks in Ventricular Myocytes

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The geometry of a dyadic calcium release unit (CRU) plays a critical role in regulating intracellular Ca^{2+} spark generation and furthermore the excitation-contraction (E-C) coupling in ventricular myocytes. A number of computational models have showed how the geometries of T-tubules and junctional sarcoplasmic reticulum (jSR) and distributions of L-type Ca^{2+} channels (LCCs) and ryanodine receptors (RyRs) could affect local Ca^{2+} signaling, but almost all of them were based on simple domain geometries such as rectangular or cylindrical shapes. For this reason, incorporating image-based realistic geometric models into mathematical simulation is timely and expected to provide more accurate simulation of many biological processes, in particular, the Ca^{2+} spark formation in ventricular myocytes.

The present study has two goals. First, we adopt a chain of image and geometric processing approaches to construct realistic 3D models (represented by high-quality surface and volumetric meshes) of both T-tubules and junctional SR from electron microscopy images of adult mouse cardiomyocytes. The distributions of LCCs and RyRs are obtained in two ways: random assignment and image-based localization (for RyRs only). The second goal of the present study is the use of Monte Carlo methods to model the randomness of release and diffusion of individual Ca^{2+} ions within a narrow dyadic cleft. To this end, the M-Cell software package, combined with the realistic geometries obtained in the first goal, is used to simulate how Ca^{2+} sparks are generated in a single dyad (or CRU) and how they change temporally and spatially with different geometries of membrane structures and distributions of channeling proteins.

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Markov Chain Models Of Coupled Calcium Channels: Kronecker Representations And Iterative Solution Methods

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Mathematical models of calcium release sites derived from Markov chain models of intracellular calcium channels exhibit collective gating reminiscent of the experimentally observed phenomenon of stochastic calcium excitability (i.e., calcium puffs and sparks). Calcium release site models are stochastic automata networks that involve many functional transitions, that is, the transition probabilities of each channel depend on the local calcium concentration and thus the state of the other channels. We present a Kronecker structured representation for calcium release site models and perform benchmark stationary distribution calculations using both exact and approximate iterative numerical solution techniques that leverage this structure. When it is possible to obtain an exact solution, response measures such as the number of channels in a particular state converge more quickly using the iterative numerical methods than occupation measures calculated via Monte Carlo simulation. In particular, multi-level methods provide excellent convergence with modest additional memory requirements for the Kronecker representation of calcium release site models. When an exact solution is not feasible, iterative approximate methods based on the power method may be used, with performance similar to Monte Carlo estimates. This suggests approximate methods with multi level iterative engines as a promising avenue of future research for large-scale calcium release site models.

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Ca^{2+} Sparks Generate Depolarizing STICs Causing Contraction And Asthmatic Hyperresponsiveness In Airway Smooth Muscle Cells

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Ca^{2+} sparks are well known to be essential for controlling the relaxation of cerebral artery SMCs; however, the functional importance of this local Ca^{2+} signaling in other types of SMCs remains to be determined. Thus, the aim of this study was to investigate the role of Ca^{2+} sparks in airway SMCs. Our data reveal that spontaneous Ca^{2+} sparks could activate spontaneous transient inward currents (STICs) at the resting membrane potential and spontaneous transient outward currents (STOCs) at more positive membrane potentials in mouse airway SMCs. Application of ryanodine to block ryanodine receptors (RyRs) abolished spontaneous Ca^{2+} sparks without altering the whole-cell cytosolic Ca^{2+} levels in single airway myocytes and decreased the resting muscle tension in isolated airway rings, whereas activation of RyRs with a low concentration of caffeine had opposite effects. Iberiotoxin, a selective blocker of big-conductance Ca^{2+}-activated K^+ channels, eliminated STOCs, but did not affect either spontaneous Ca^{2+} spark activity or resting muscle tension. In contrast, NPPB, an inhibitor of Cl^- channels, reduced resting muscle tension. The effect of NPPB was prevented in the presence of the selective voltage-dependent Ca^{2+} channel blocker nifedipine. We have also found that the activity of Ca^{2+} sparks in single asthmatic mouse airway SMCs and in-vivo airway resistance in asthmatic mice were significantly increased. Interestingly, ryanodine caused a stronger relaxation in asthmatic airway smooth muscle. Taken together, these findings suggest that spontaneous Ca^{2+} sparks can activate Ca^{2+}-activated Cl^- channels and then generate STICs, causing membrane depolarization, opening of voltage-dependent Ca^{2+} channels, extracellular Ca^{2+} influx and contraction in airway SMCs. Moreover, Ca^{2+} sparks and attendant STICs are both increased in asthmatic airway SMCs, which may contribute to asthmatic airway hyperresponsiveness.

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Decreased Ca^{2+} Spark Frequency and RyR2 Expression in Cerebral Arteries Following Subarachnoid Hemorrhage

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Ca^{2+} sparks represent local and transient increases in intracellular Ca^{2+} caused by the coordinated opening of ryanodine receptors (RyRs) located in the sarcoplasmic reticulum of muscle. Contrary to the contraction caused by global Ca^{2+} increases, Ca^{2+} sparks promote smooth muscle relaxation via activation of plasmalemmal large-conductance Ca^{2+}-activated potassium (BK) channels leading to membrane potential hyperpolarization and decreased activity of voltage-dependent Ca^{2+} channels. Here, we examined whether impairment of this vasodilator pathway contributes to enhanced cerebral artery constriction associated with subarachnoid hemorrhage (SAH). Using a rabbit SAH model, Ca^{2+}

sparks, single channel and whole-cell BK currents were measured in cerebral artery myocytes. Expression of BK channel alpha and beta-1 subunits and RyR2 was examined by RT-PCR. The effects of blockers for BK channels (paxilline, 1 μM) and RyRs (ryanodine, 10 μM) were examined on diameter of isolated cerebral arteries. Ca^{2+} spark frequency, but not amplitude, was decreased ~50% following SAH. This decrease in Ca^{2+} spark frequency corresponded to a reduction in the number of functional Ca^{2+} spark sites and decreased RyR2 expression in myocytes from SAH animals. A similar reduction in the frequency of transient BK currents was observed following SAH, although the properties and expression of BK channels were similar between groups. Inhibition of this vasodilatory pathway by paxilline or ryanodine induced constriction of control arteries, which was greatly diminished following SAH. These data suggest decreased Ca^{2+} spark frequency in cerebral myocytes following SAH is due to decreased RyR2 expression and a reduction in functional spark sites. The resulting decrease in BK currents leads to an enhanced cerebral artery constriction that may contribute to the development of neurological deficits following SAH. (Supported by AHA 0725837T, 0725841T, NIH R01 HL078983, R01 HL44455, and the Totman Medical Research Trust).

1421-Pos Board B265

Mitochondrial Modulation Of Spontaneous Ca^{2+} Oscillations In Portal Vein Smooth Muscle Myocytes

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Transient increases in cytosolic Ca^{2+} concentration ($[Ca^{2+}]_c$) regulate many smooth muscle activities including contraction, transcription, growth and apoptosis. The onset of some processes involve sarcoplasmic reticulum Ca^{2+} release triggered by either inositol 1,4,5-trisphosphate ($InsP_3$) or occurring spontaneously. Mitochondrial Ca^{2+} uptake influences the spatio-temporal features of spontaneous and $InsP_3$-mediated Ca^{2+} waves. For example the magnitude of phenylephrine-evoked Ca^{2+} waves was decreased when mitochondrial membrane potential ($\Delta\Psi_M$) was depolarized. How individual mitochondria interact to regulate global Ca^{2+} signals is unresolved. Mitochondria may act as a series of separate entities or as a continuum to shape the amplitude, propagation speed and frequency of these Ca^{2+} release events. We examined how mitochondria affect spontaneous Ca^{2+} oscillations by either inhibiting mitochondrial Ca^{2+} uptake in small isolated regions or throughout the entire cell. Mitochondrial Ca^{2+} uptake was inhibited throughout the entire cell by depolarizing the $\Delta\Psi_M$ using the protonophore CCCP to dissipate the driving force for Ca^{2+} uptake. Alternatively, mitochondrial Ca^{2+} uptake was inhibited in small, restricted regions by locally photolyzing a caged mitochondrial uncoupler (caged AG10), which we have synthesised. Photolysis of caged AG10, in a small region depolarizes $\Delta\Psi_M$ in that area alone leaving the $\Delta\Psi_M$ of the remaining mitochondrial complement intact. Spontaneous Ca^{2+} oscillations were observed in about 25% of the cells examined. When $\Delta\Psi_M$ was depolarized throughout the cell oscillations were inhibited. In contrast, depolarization of $\Delta\Psi_M$ in a small isolated region of the cell decreased the amplitude but increased the frequency of Ca^{2+} oscillations. These results indicate that mitochondria act as independent entities but their activities throughout coordinate to regulate Ca^{2+} release. Supported by the Wellcome Trust, British Heart Foundation, BBSRC and Leverhulme Trust.

1422-Pos Board B266

Dopamine and Pancreatic Islet Function

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Pancreatic islets are spheroidal clusters of electrically and chemically connected endocrine cells, whose function plays a key role in glucose homeostasis. Islet β-cells secrete insulin in response to increased blood glucose concentration, but their response is modulated by other stimuli (such as glucagon, insulin, acetylcholine, epinephrine, etc.). The application of stimulatory glucose concentrations (>6 mM) to isolated pancreatic islets produces synchronized oscillations in intracellular $[Ca^{2+}]$. During these $[Ca^{2+}]$ oscillations, the entire pancreatic islet secretes insulin in a pulsatile manner.

We are exploring the role of endogenous production of dopamine by β-cells in the regulation of $[Ca^{2+}]$ oscillations and insulin secretion. Using fluorescent Ca^{2+} indicators, micro-fluidic devices, and confocal microscopy, we have measured the period of $[Ca^{2+}]$ oscillations in isolated intact islets. Treating the islets with the dopamine precursor, L-DOPA (3,4-Dihydroxy-L-phenylalanine), or a selective antagonist of D2 dopamine receptor, Raclopride, causes a decrease and an increase in the frequency of $[Ca^{2+}]$ oscillations. Parallel experiments were performed using islets from genetically modified mice that do not express the dopamine transporter, and it was found that their $[Ca^{2+}]$ oscillations are also slower than those from wild type mice. Hence, we propose that this autocrine dopaminergic system is part of the mechanism that regulates $[Ca^{2+}]$ oscillations.

The oscillation period depends on the islet electrical and metabolic activity. To begin understanding the potential mechanisms by which dopamine is exerting its function in the β-cell, we are using computer modeling to simulate the islet activity and reproduce the data and to gather information on the main pathway involved in the observed dopaminergic effect.

1423-Pos Board B267

Interactions With Imidazole Side Groups As A Mechanism To Block The Alzheimer'S Aβ Peptide-induced Intracellular Calcium Increase

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We have proposed the formation of Aβ ion channels as a mechanism to explain the intracellular calcium increase which occurs after cells are exposed to the cytotoxic Alzheimer's Aβ peptide. We developed highly effective and specific histidine-related Aβ channel blockers which prevent the Aβ-induced intracellular calcium response. Substitution experiments showed that histidine is essential for the blocking process. We hypothesized that the blocking efficiency of these compounds was related to imidazole side chains in the histidine residues. We rationalized that the resonance structure of the imidazole ring would be attracted to a full or partial positively charged form of the Histidine in the Aβ subunits of the Aβ channels. This investigation studied the intracellular calcium increase which occurs after cells are exposed to the Aβ peptide. The role of the imidazole side chains in mechanism of action was determined using histidine-related Aβ channel blockers in which the imidazole side chains were methylated. Additionally, we studied these compounds when modified by amidation and acetylation of the carboxyl and amine end groups, respectively. Our results showed that the efficacy to prevent the Aβ-induced intracellular calcium increase, and the capacity to protect cells from the toxic action of Aβ, is completely abolished when the imidazole side chains of the histidine-related Aβ channel blockers are methylated. On the other hand, the efficacy of the Aβ channel blockers is significantly improved when the ends of the molecules are capped leaving the imidazole side chain as the sole group available for interaction. We conclude that aromatic interactions between the imidazole side chains in the histidine-related blockers, and the charged form of the His residues in the Aβ channels, constitutes a blocking mechanism for the Aβ peptide-induced intracellular calcium increase.

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Tightly Regulated Store-operated Ca^{2+} Entry In Healthy And Dystrophic Skeletal Muscle

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Store-operated Ca^{2+} entry (SOCE) is a mechanism that allows the entry of extracellular Ca^{2+} upon depletion of the internal stores. This mechanism has been described in skeletal muscle and the two main molecular players, Stim1 and Orai1, have been identified. Previous work, mainly on myotubes, suggested that SOCE may become deregulated in dystrophic skeletal muscle and result in cellular Ca^{2+} overload. The final result of such a process would be activation of proteolytic enzymes, cell necrosis and/or apoptosis. To examine the regulation of SOCE in healthy and dystrophic muscle we examined the biochemistry and physiology of skeletal muscle from wild-type (wt) and mdx mice (8-20 weeks old). Western blotting of single fibres showed that Stim1 and Orai1 were expressed at 2-3 times higher levels in mdx compared with wt muscle (normalized to total myosin). Consistent with this, enzymatically isolated interossei fibres loaded with fluo-4AM and depleted of Ca^{2+} in a solution containing 0 Ca^{2+}, 20 μM cyclopiazonic acid and 10 mM caffeine showed a 3-fold higher rate of Ca^{2+} entry into the depleted mdx fibres compared to wt upon re-addition of 2 mM Ca^{2+}. However, this does not imply Ca^{2+} overload will occur via SOCE in dystrophic cells if deactivation is unaffected. To study SOCE kinetics, skinned fibres with t-system trapped fluo-5N were bathed in an internal solution with rhod-2 and continuously imaged in xyt mode on a confocal microscope. Intracellular Ca^{2+} release was induced by lowering cytoplasmic $[Mg^{2+}]$. Transient t-system Ca^{2+}-depletion and reuptake was preceded by transient SR Ca^{2+}-release and reuptake in fibres from both mdx and wt mice. This indicates robust activation and deactivation mechanisms of SOCE in both wt and dystrophic muscle which prevent not only depletion but also overloading of the internal Ca^{2+}-stores.