1552-Pos
Deriving Cellular Ca(2+) Dynamics from Puff Characteristics - A New Approach in Mathematical Modeling
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It has recently been shown that Ca(2+) spikes are stochastic and that the Ca(2+) concentration is subject to huge intracellular gradients. These findings underline the need for stochastic models taking single molecule state transitions into account (Master Equations are impossible due to the high number of states). We developed such a modeling concept based on the fact that for cellular concentration dynamics, it is only relevant whether channels are open or closed, but not in which open or closed state they are. That requires a non-Markovian formulation of the theory which we will present. This formulation permits direct integration of measurable quantities into the model, whereas detailed knowledge of physiologic parameters is not necessary. Therefore, input and output can be directly compared with experiments, if we consider observables like the probability that no spike happens or the average spike length. The model covers experimentally observed modes of system behavior like bursting, spiking or local events. Statistic properties of interspike intervals are in good accordance with experimental data. Since we have developed an analytic description as well as an efficient simulation algorithm, we are in a position to analyze a broad range of possible system configurations based on a solid theoretical background. In particular, we find that the spiking pattern can be predicted merely from two parameters, the cluster coupling and the average channel open time. The latter is probably constant and the former can be regulated by second messengers or by the number of active clusters, e. g. This implies that local dynamics emerges to a single control parameter determining global dynamics. Since we expect similar mechanisms to work in various fields of biology (e.g. cAMP dynamics and DNA replication), our theory should be widely applicable.

1553-Pos
Simulations of Calcium Oscillations in Pancreatic Acinar Cells
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Pancreatic acinar cells have developed sophisticated Ca^{2+} signaling mechanisms, which have important physiological roles on enzyme and fluid secretion. In response to stimulation, Ca^{2+}signals in pancreatic acinar cells are modulated in a precise spatiotemporal manner necessary for normal secretory function and are generally confined to the apical pole. Meanwhile, abnormal, prolonged global elevation of cytosolic Ca^{2+} concentration has been proposed to be the crucial trigger for pancreatitis. In this study, we have constructed and validated a computational model to gain insights into the generation of different Ca^{2+} signaling patterns in pancreatic acinar cell. Specifically this model allows us to examine the roles of and interactions between inhomogeneous distributed IP_3 and rynodine receptors, the passive and active roles of the mitochondrial barrier that separates the apical and basal regions, and dimensionality. Preliminary results indicate that both the topology of the mitochondrial barrier and mitochondrial Ca^{2+} uptake have significant impact in both confining and maintaining the apical oscillations, supporting the idea that mitochondria are active and crucial elements of Ca^{2+} signaling in pancreatic acinar cells. In addition, when using the same parameters for the kinetics, changing from a two-dimensional to three-dimensional model of the cell yields qualitatively different results for the apical Ca^{2+} oscillation.

1554-Pos
Calcium Dynamics in Cardiac Mitochondria
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Mitochondria play a significant role in the metabolic control of cardiac ventricular myocytes. They not only are the dominant intracellular organelle (~35% of the intracellular volume) but they have been reported to influence: intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$)locally and dynamically, cellular apoptosis and cellular function. Many of the specific findings, however, are in dispute. We have used the high affinity calcium indicator Rhod2 to measure mitochondrial Ca^{2+} in permeabilized cells. (Fig 1). We do observe changes in mitochondrial Ca^{2+} ($[Ca^{2+}]_{mito}$) when $[Ca^{2+}]_i$ changes but these changes are modest.

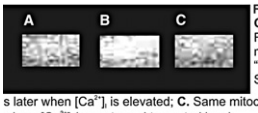

Fig. 1. Mitochondrial Calcium increase. A. Rhod2-loaded single mitochondria under "control" conditions ; **B.** Same mitochondrion 13 s later when $[Ca^{2+}]_i$ is elevated; **C.** Same mitochondrion 17 s after B when $[Ca^{2+}]_i$ has returned to control level.

Ryanodine Receptors I

1555-Pos
Biophysical and Cellular Characterisation of Alternatively Spliced Isoforms of the Human Cardiac Ryanodine Receptor (RyR2)
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Developmentally regulated and tissue-specific alternative splicing of the human cardiac ryanodine receptor (RyR2) may represent an innate mechanism for tuning intracellular Ca^{2+} handling. The precise molecular basis of channel modulation by alternative splicing (30bp and 24bp inserts encoding FAIDSLCGFG and VTGSQRSK, respectively) remains unknown. To further explore the mechanistic basis of altered RyR2 function by alternative splicing we expressed and characterised recombinant human RyR2 channels. We measured the Ca^{2+} activation of detergent-free homotetrameric channels using an *in vitro* ryanodine binding assay and showed that $RyR2^{24+}$ exhibited Ca^{2+}-sensitive ryanodine binding (EC_{50} $0.65 \pm 0.01\mu M$; B_{max} at ambient $[Ca^{2+}] > 5\mu M$, 380 ± 48 fmol/mg) that was entirely comparable to channels formed from 'unspliced' RyR2 subunits (EC_{50} $0.52 \pm 0.17\mu M$; B_{max} 350 ± 33 fmol/mg). In marked contrast, $RyR2^{30+}$, the predominant isoform in human embryonic hearts, did not exhibit Ca^{2+}-sensitive ryanodine interaction at equilibrium and binding was greatly reduced even at $[Ca^{2+}]$ up to 0.5mM (B_{max} 56 ± 5 fmol/mg). RyR2 mutations in the putative Ca^{2+} pore-lining helix abolish RyR2 interaction with ryanodine in 'steady state' assays (Wang et al., JBC 278 2003). Our finding that insertion of the FAIDSCLCGFG sequence distal to amino acid 1477 in $RyR2^{30+}$ ablates Ca^{2+}-sensitive ryanodine binding suggests that long range conformational interactions within the polypeptide may regulate the interaction with ryanodine and/or the Ca^{2+} sensitivity of the channel. The precise kinetics of ryanodine interaction with channels purified using sucrose density gradients were determined in single channel lipid bilayer experiments using K^+ as the permeant ion. The effects of synthetic peptides corresponding to the 30bp and 24bp splice epitopes on channel functionality *in vitro* and in living cardiac cell-based assays were also studied. Our data provides novel insights into the mode of RyR2 channel regulation by alternative splicing.

1556-Pos
Stabilisation of Ryanodine Binding to RyR2 - How Many Monomers are Involved?
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An important contribution to our ability to study structure and function of the Ryanodine Receptor (RyR) Ca^{2+} release channel was the discovery of the plant alkaloid ryanodine, which binds to the channel only in the open state. Indeed the [^{3}H]-ryanodine binding assay is a widely utilised experimental technique and provides quantifiable information regarding the density and functional properties of a population of RyRs. Despite this, the precise molecular basis of the ryanodine-RyR interaction remains largely undefined. The inner helix of the pore of the cardiac mouse RyR (mRyR2) has been proposed as crucial for this interaction, and the mutation Q4863A has been shown to abolish ryanodine binding (at equilibrium) but is still functional in all other respects i.e. Ca^{2+} release in cells and at the single channel level (J Biol Chem 2003, 278, 51557-51565). The Q4863A mutation destabilises ryanodine once it is bound at the high affinity site (Mol Membr Biol 2007, 24, 185-93). Our aim is to use this mutant to establish the number of wild-type (WT) mRyR2 monomers necessary to stabilise bound ryanodine in the tetramer.
Assuming that WT and Q4863A monomers associate equally, the probable occurrence of each homo/heterotetramer type can be calculated using binomial theory, and subsequently the [^{3}H]-ryanodine binding level of each predicted if 1,2,3 or all 4 WT monomers are required to stabilise the interaction. In practice, ryanodine stabilisation has been determined by measuring [^{3}H]-ryanodine binding to mixed heterotetramer populations of mRyR2 under maximally activated conditions following co-expression of WT and Q4863A monomers in differnt ratios in HEK293 cells. Initial results show that 2 WT monomers are required for high affinity binding. The mechanisms governing binding will be investigated at the single channel level. Supported by the British Heart Foundation and HSFA.