The Effect of Bioenergetic Impairment of Cytosolic Processes in Spatio-Temporal Ca²⁺ Dynamics in a Three-Dimensional Cardiomyocyte Model

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Abstract

The heart consumes large amounts of energy with each beat. Mitochondria are the source of over 95% of this energy in the form of ATP and rely on increased Ca^{2+} uptake to stimulate production in times of increased work. Ca^{2+} uptake into the mitochondria primarily occurs within microdomains. Structural remodeling associated with heart failure may disrupt these microdomains leading to impaired mitochondrial Ca^{2+} uptake and energetic impairment.

To investigate the effect of structural changes on single cell behavior, a model describing mitochondrial dynamics and energetics production is modified and incorporated into a recently developed three-dimensional model of spatio-temporal calcium handling, which preserves microdomain structure and incorporates stochastic processes in Ca^{2+} handling protein kinetics. Modifications to the mitochondria model included a reformulation of mitochondrial Ca^{2+} uniporter uptake, making it suitable for concentrations in microdomains.

With this model we demonstrate the importance of an ordered structure within the cell for normal function. Changes in the arrangement of mitochondria can have a pronounced effect on intracellular Ca^{2+} dynamics through their energetic regulation of SERCA, leading to spatially heterogeneous sarcoplasmic reticulum uptake and loading.

1. Introduction

Heart disease is a leading cause of mortality in the developed world and is responsible for almost 20% of all deaths, with a million people in the United Kingdom also suffering from heart failure (HF) [1]. With HF comes an increased risk of arrhythmogenesis correlated with structural and electrophysiological remodelling of the heart. The underlying mechanisms of these arrhythmias have not been fully elucidated, and computational modelling can play a role in development of a greater understanding of the arrhythmogenic substrate.

Modern imaging techniques have shown the complex structure of the cardiomyocyte and how it remodels with disease [2]. These methods are revealing the structure of single cells at high resolution, allowing structures such as the sarcoplasmic reticulum (SR) network to be imaged in detail. With access to this high resolution data we may begin to understand how localization of organelles in the cell affects their function. It may also be possible to assess how changes in structural integrity of the interfaces between organelles such as the junctional sarcoplasmic reticulum (jSR), t-tubules and the mitochondria, labelled the Ca²⁺ microdomain, affect cellular function [3].

Over 95% of the ATP consumed by the cell is produced by mitochondria which line the inner membrane and the myofibrils [4]. ATP production is closely regulated through Ca²⁺ stimulation of the Kreb's cycle within the mitochondria. To stimulate the production of ATP Ca²⁺ must be carried into the mitochondrial matrix by the mitochondrial Ca²⁺ uniporter (MCU) [3]. As the MCU has a low affinity to Ca²⁺, microdomains are formed of a t-tubule, junction SR and a mitochondrion (see Figure 1). Within these microdomains the Ca²⁺ concentration may be an order of magnitude higher than the bulk cytosol. ATP produced by the mitochondria may then be used by the myofibrils and cytosolic ATPases such as the sarcoplasmic reticulum Ca²⁺-ATPase (SERCA). SERCA is crucial for Ca²⁺ dynamics within the cell and is sensitive to changes in bioenergetic status. Such changes may modulate Ca²⁺ cycling and in turn electrical behaviour.

In healthy cells the SR, t-tubules and mitochondria are observed to be intertwined allowing normal Ca²⁺ movement between the organelles. With disease this order is disrupted with a breakdown of the SR and loss of t-tubules. Mitochondria, which are seen in lines along the myofibrils, are found to cluster and empty spaces are observed where mitochondria once were. The t-tubules are invaginations carrying Ca²⁺ deep within the cell, and thus their loss can lead to a reduction in the order of Ca²⁺ induced Ca²⁺ release. Microdomain structure, which may be important for sufficient mitochondrial Ca²⁺ uptake, is

observed to breakdown and levels of tethering proteins that hold the jSR and mitochondria together are found to be reduced in HF. With HF increase in levels of β-adrenergic stimulation are also observed. Through phosphorylation of targets on the L-type Ca^{2+} channel and the ryanodine receptors (RyRs) their open probabilities are increased. SERCA uptake is also increased.

How these structural changes affect mitochondrial ATP production, Ca^{2+} cycling and electrical behaviour is incompletely understood. Here we investigate how changes in the spatial behaviour of Ca^{2+} cycling can affect whole cell dynamics to see if they underlie any changes in electrical behaviour that may lead to arrhythmogenesis.

2. Methods

2.1. Cell model

For this study a previously developed model of spatio-temporal Ca^{2+} cycling and electrophysiology was modified to incorporate a model of bioenergetics and force production [5]–[7]. The 3D model was constructed of 20,000 compartments each containing a Ca^{2+} release unit (CRU) and associated mitochondria. Each CRU consists of L-type Ca^{2+} channels, Na^+/Ca^{2+} exchanger and RyR receptors (Figure 2). To model cellular bioenergetics the Gauthier *et al.* model was modified for Ca^{2+} uptake from the dyadic space in which concentrations are higher than that of the bulk cytosol [6]. The open probability of the mitochondrial Ca^{2+} uniporter was shifted for a higher open probability at higher concentrations based on recent data [8].

To allow us to simulate the effect of changes in bioenergetics on intracellular Ca^{2+} dynamics, regulation of SERCA in each compartment was linked to local ATP and ADP concentrations. Ca^{2+} uptake and efflux from the mitochondria was linked to that of the dyadic space.

For simulation of the levels of ATP hydrolysis by actomyosin ATPase, the Tran *et al.* model of force production was incorporated [7]. Regulation of force production was linked to the average cytosolic Ca^{2+} concentration and the model linked to the bioenergetics model through whole cell average ATP/ADP concentrations.

2.2. Structural remodelling

Simulating mitochondrial clustering was achieved through a 90% block of the MCU in select patches of the cell. This block was made on the assumption that with clustering mitochondria lose their privileged position within the Ca^{2+} microdomain (Figure 1). For these simulations the model was paced until the mitochondrial model reached a steady state and then mitochondrial Ca^{2+} uptake was blocked. Detubulation was simulated through

the removal of I_{CaL} and I_{NCX} currents within specific compartments.

2.3. β-adrenergic stimulation

To simulate the effects of β-adrenergic stimulation on the cell the open probability of the RyRs was increased by 30% from control. L-type Ca^{2+} channel opening was increased by 25% and SERCA uptake by 200%.

2.4. Ca^{2+} diffusion within the nSR

As measurements of the diffusion of Ca^{2+} within the network SR differ largely three simulations were run with different time constants of diffusion to examine how changes in this value affect the propagation of Ca^{2+} through the cell. Propagation of Ca^{2+} waves was also observed with each simulation. With breakdown of the ordered structure of the SR with HF this may be simulated through the increase of the time constant of diffusion throughout the SR [2].
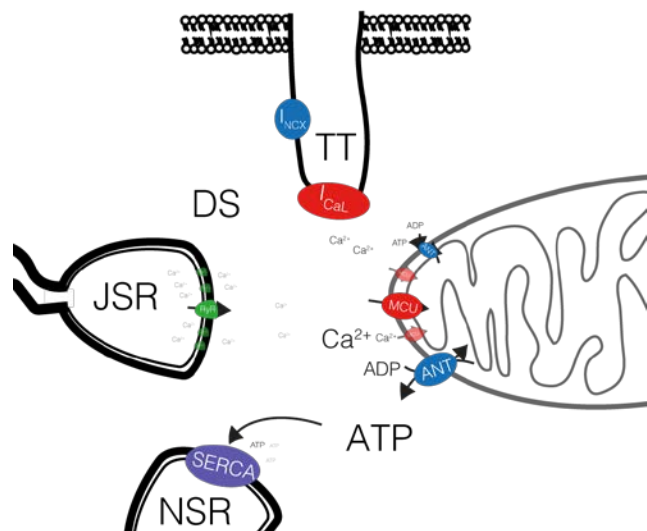

Figure 1. Schematic of Ca^{2+} **microdomain.**
The junction at which a t-tubule, mitochondrion and the junctional sarcoplasmic reticulum meet. ATP is transported from the mitochondria and is taken up by SERCA.
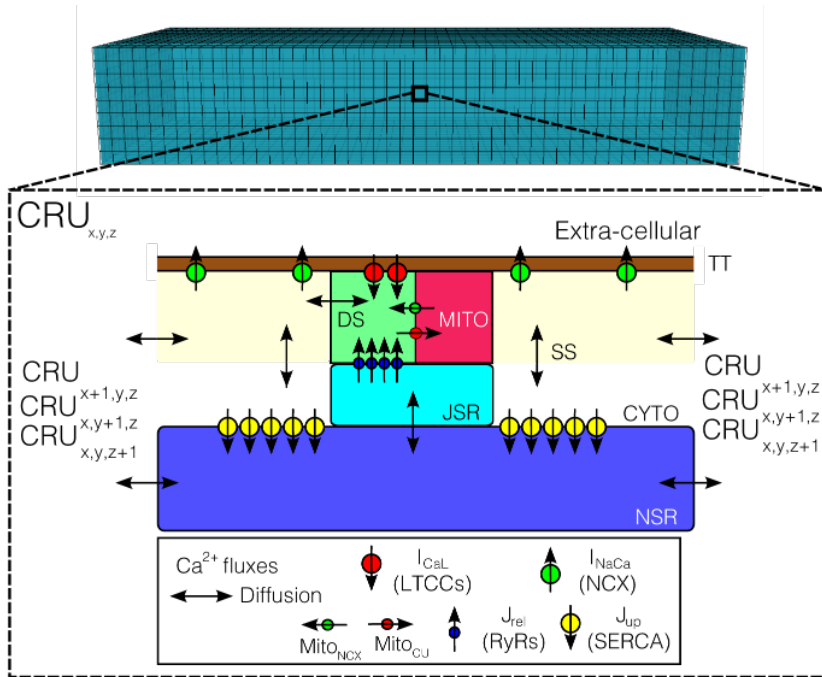
Figure 2. Schematic of cell with CRUs in a 3D grid. Each CRU is coupled compartmentally. Ca^{2+} transport mechanisms are shown by arrows and coloured circles. Mitochondrial Ca^{2+} uptake through the mitochondrial Ca^{2+} uniporter is taken from the dyadic space compartment. With efflux from the mitochondria back into dyadic space compartment. CRU, Ca^{2+} release unit; DS, dyadic space; MITO, mitochondria; JSR; junctional SR; CYTO, cytoplasm; TT, t-tubule; NSR, network SR.

3.   Results

3.1.   Effect of mitochondrial clustering

In the patches of mitochondria with the mitochondrial Ca^{2+} uniporter blocked, lower levels of mitochondrial matrix Ca^{2+} was observed. This resulted in a decrease in Kreb's cycle enzyme activity and lower ATP production. ATP production in these patches was found to be considerably lower than in compartments with normal mitochondrial Ca^{2+} uptake. Due to this reduction in ATP concentrations within these compartments, SERCA was impaired. A large reduction in SERCA uptake results in higher cytosolic Ca^{2+} concentration and a lower SR concentration. Gradients in cytosolic Ca^{2+} concentration are illustrated in Figure 3.

3.2.   Detubulation in HF

With detubulation, a loss of Ca^{2+} influx is observed due to loss of L-type Ca^{2+} channels in those compartments. This reduction is accompanied by a reduction in Ca^{2+} release from the RyR that rely on I_{CaL} for activation. With this reduction in dyadic Ca^{2+} concentration, we observe a reduction in local energetic availability due to lack of stimulation of ATP production. SERCA impairment then leads to a further increase in Ca^{2+} gradient between compartments within the cell.

With faster pacing at a BCL of 400 ms there was a reduction in pumping time for SERCA. This resulted in impaired SR filling and greater Ca^{2+} gradients within the cell leading to spatial Ca^{2+} alternans (Figure 4).
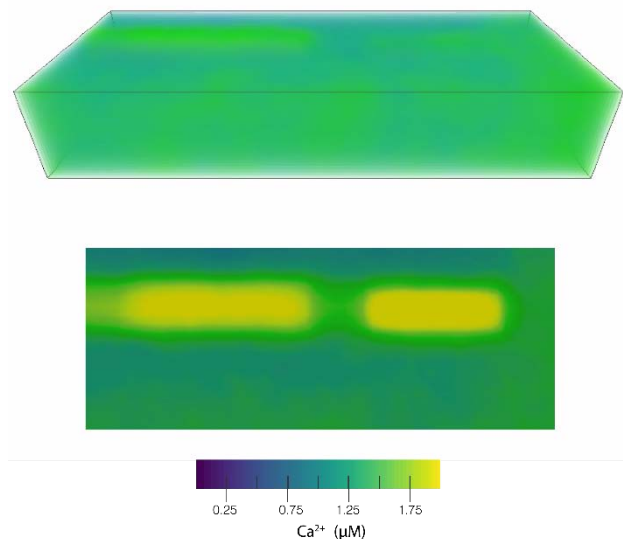

Figure 3. 3D and 2D slice of cytosolic Ca^{2+}. 50 ms after stimulus, during the decay phase of the Ca^{2+} transient, the patches in which mitochondrial Ca^{2+} uniporter has been blocked can be clearly seen.
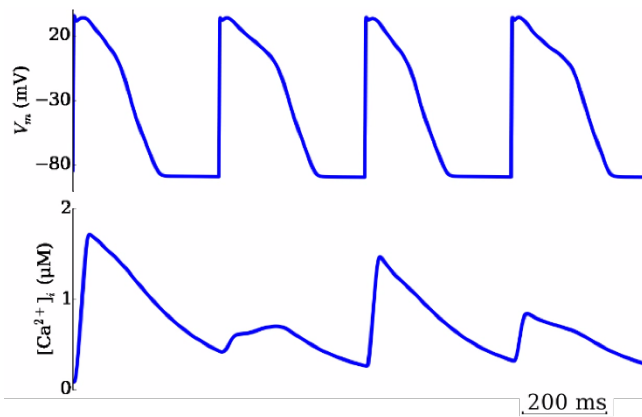
Figure 4. Ca^{2+} **alternans due to t-tubule remodeling and impaired SERCA function.** A reduction in pumping time and reduced total SERCA uptake leads to alternans.

3.3. β-adrenergic stimulation

In Figure 5 a Ca^{2+} wave originating within a patch of MCU blocked mitochondria can be seen. A Ca^{2+} wave is produced from a patch in which MCU is blocked due to SR and Ca^{2+} gradients. A Ca^{2+} spark can also be observed in the other highlighted region, though in this case it did not lead to a Ca^{2+} wave. An increase in Ca^{2+} waves is seen with heart failure and with an increase in β-adrenergic stimulation [9], [10].
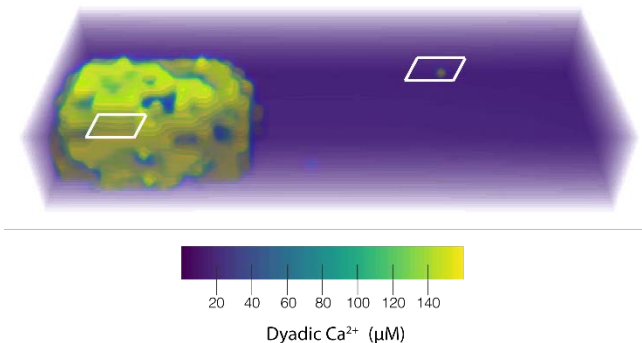

Figure 5. Ca^{2+} wave and Ca^{2+} spark originating at sites of MCU block. Areas highlighted are sites of MCU block.

4. Conclusion

In this study we present a three-dimensional model that is capable of simulating bioenergetically regulated spatio-temporal Ca^{2+} dynamics. We show energetic compromise of intracellular processes can lead to changes in cell Ca^{2+} dynamics and whole cell behavior.

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