Developing a Cardiac Calcium Handling Model with Integrated Lysosomal and Mitochondrial Dynamics

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Abstract

Intracellular calcium dysregulation contributes to impaired contraction and arrhythmogenesis, yet most cardiac cell models focus mainly on sarcolemmal and sarcoplasmic reticulum fluxes. Mitochondria and lysosomes are increasingly recognised as active calcium-handling organelles, but their combined role in excitation-contraction coupling remains unclear. This is particularly true for lysosomes, whose function in cardiomyocyte calcium handling is poorly characterised with many unknowns.

Here, we integrate lysosomal and mitochondrial calcium dynamics into human cardiac cell models, including T-World and a human induced pluripotent stem cell-derived cardiomyocyte (iPSC-CM) model. Published lysosomal and mitochondrial formulations are incorporated into calcium handling. We also explore translation toward a submicron calcium handling model informed by ryanodine receptor structural data.

Under baseline pacing, integrated models preserve stable excitation-contraction coupling and show organelle-cytosolic calcium coupling, with limited effects on the global calcium transient. These models provide a framework for investigating how lysosomal and mitochondrial dysfunction may modulate calcium cycling during energetic impairment or structural remodelling.

1. Introduction

Calcium-induced calcium release (CICR) underlies excitation-contraction coupling in cardiomyocytes. Membrane depolarization activates L-type calcium channels (LTCC), producing a local increase in calcium concentration that activates clusters of type-2 ryanodine receptors (RyR) and initiate calcium release from the sarcoplasmic reticulum (SR). Perturbations to this system can generate spontaneous calcium release, calcium waves, alternans and triggered activity. Consequently, computational models of cardiac electrophysiology typically provide more or less detailed representations of currents in the cell membrane or through cytosol and SR and intracellular calcium buffering.

However, calcium regulation within cardiomyocytes is not restricted to the cytosol and SR. Mitochondria exchange calcium with the cytosol through mechanisms including the mitochondrial calcium uniporter (MCU), mitochondrial sodium-calcium exchange and the mitochondrial permeability transition pore (mPTP). This is not all the extent of mitochondrial activity, since it can also influence excitation-contraction coupling (ECC) via its coupling through ATP availability or reactive oxygen species (ROS). Under physiological conditions, mitochondrial calcium handling may have only modest effects on the bulk cytosolic calcium transient, although some studies suggest that mitochondria might promote calcium waves under some circumstances.

Lysosomes represent a second, substantially less studied calcium store. Calcium uptake into lysosomes and release through two-pore channels (TPC) may provide a rapid calcium-transport pathway that alters cytosolic and junctional calcium concentrations. Some studies suggest that lysosomal calcium loading and release can affect RyR activity and the likelihood of spontaneous calcium release, particularly during calcium overload.

Here, we integrate lysosomal and mitochondrial calcium handling into the T-World human ventricular model [1] and the human iPSC-CM model developed by Li, Forouzandehmehr *et al* [2]. We also incorporate discrete mitochondria and lysosomes into a submicron spatial model containing experimentally derived RyR cluster structures. We evaluate organelle effects under baseline conditions and mitochondrial depolarization, and examine whether mitochondria-RyR separation modifies local calcium dynamics.

2. Methods

2.1. Baseline whole-cell models

Organelle calcium handling is incorporated into the T-World human ventricular model and the Li-Forouzandehmehr human iPSC-CM model (Fig. 1A). Both models include membrane electrophysiology, calcium cycling and contraction. The original formulations are retained except for coupling to organelle calcium, ATP and ROS.

2.2. Lysosomal and mitochondrial calcium handling

Lysosomal calcium handling is adapted from Meng *et al* [3] and includes uptake from cytosol, passive leak and TPC-mediated release. In T-World, lysosomal release is coupled to both bulk and junctional calcium. Because the iPSC-CM model has no junctional compartment, the lysosomal flux is fully assigned to the bulk cytosol.

Mitochondrial calcium and signalling dynamics are adapted from the spatial ventricular myocyte model of Song *et al* [4]. This model includes mitochondrial calcium uptake through the MCU, extrusion through mitochondrial $Na^+ - Ca^{2+}$ exchange, stochastic mPTP opening, membrane potential dynamics and ROS- and ATP-dependent signalling and dynamics. The original spatial model was formulated as a 3D network of calcium release units coupled to spatially distributed mitochondria. For incorporation into non-spatial whole-cell models, the original formulation is converted into a whole-cell representation corresponding to approximately 7000 mitochondria. ATP-dependent modulation is applied to SERCA and the Na^+/K^+ pump, while ROS-dependent modulation is applied to RyR activity and SERCA uptake.

As with lysosomes, mitochondrial calcium exchange can be distributed between the bulk cytosolic and junctional compartments. In the iPSC-CM model, all mitochondrial exchange is assigned to the cytosol. Moreover, the ROS-dependent modulation of RyR needs a different treatment. Since the iPSC-CM model presents Hodgkin-Huxley-type gating rather than state-transition rates, ROS-dependent enhancement is represented by reducing the RyR activation time constant.

2.3. Submicron spatial model

Spatial simulations are based on the submicron framework developed by Marchena *et al* [5], which describes calcium diffusion and buffering in cytosolic and SR domains using effective concentration fields and was originally developed to study calcium sparks and the effect of RyR cluster organization. The model has been extended

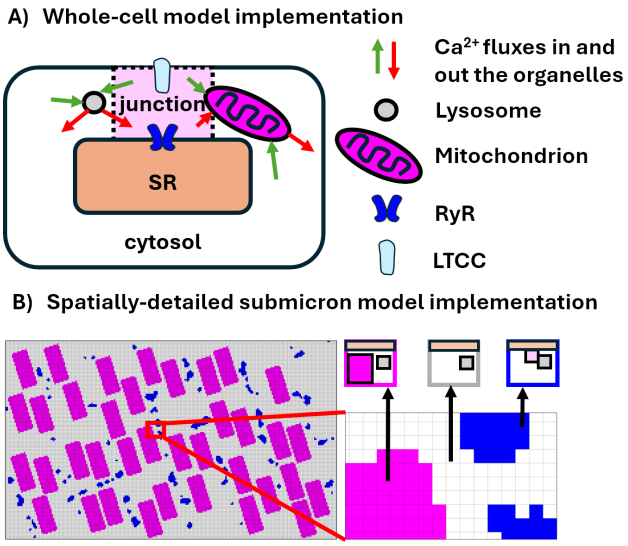

Figure 1. Schematic representation of lysosomes and mitochondria implementation in whole cell (A) and spatially-detailed submicron model (B).

to include additional calcium compartments and heterogeneous sources operating at different spatial resolutions. The simulated domain represents a small section of a cardiomyocyte divided in pixels of approximately 37×37 nm (Fig. 1B). RyR cluster geometry was obtained from Hurley *et al* [6]. LTCC clusters are considered colocated with the RyR clusters, hence located in the same pixels, while SERCA and NCX are distributed throughout the domain.

LTCCs and RyRs are represented at their cluster resolutions, while cytosolic and SR calcium are solved on the pixel grid level. Exchange between these representations is implemented by local coarse-grained coupling. Fluxes generated by these channels are distributed to the overlapping pixels and the calcium concentration sensed by each structure is calculated as a local spatial average over the corresponding coupling region.

Discrete mitochondria are generated between Z-lines. While RyR clusters are predominantly located in Z-line regions, mitochondrial staining shows limited colocalization with RyR clusters [7]. Mitochondria are represented as independent objects with dimensions of approximately 1 micrometer long and 0.45 micrometers wide. These are treated the same way as RyR and LTCC clusters, this is, at whole-mitochondrion level coupling to the pixel grid of cytosol. Thus, intra-mitochondrial calcium diffusion is not considered and, due to the lack of colocalization with RyR, then with junctional space, only the exchange with cytosol is simulated. ATP diffuses throughout the cytosol, with production restricted to mitochondrial pixels. ROS is represented by a fixed spatial profile that decreases with distance from mitochondria. Simulations are performed

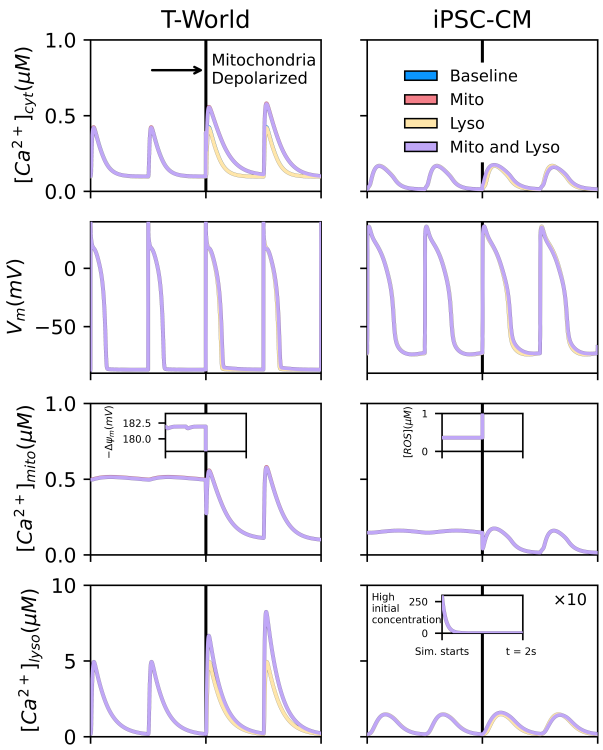

Figure 2. Cytosolic, mitochondrial and lysosomal calcium concentrations and membrane voltage in the whole-cell models with lysosomes, mitochondria, both organelles or neither. Insets show mitochondrial membrane potential in T-World, ROS in the iPSC-CM model and initial lysosomal depletion. iPSC-CM lysosomal calcium is scaled by a factor of 10 for visualization.

using minimum mitochondria-RyR separations of 50 and 200 nm.

Because the spatial distribution of lysosomes is less well characterised, lysosomal calcium is represented in each pixel using a uniform total volume fraction of 2%. Each pixel contains its own lysosomal calcium state which is exchanged with the associated cytosolic calcium and, if applicable, also the junctional.

To actively initiate CICR, LTCC are activated under a prescribed voltage-clamp waveform. The applied voltage follows the formulation of Shiferaw *et al* [8] at 1Hz pacing.

3. Results

3.1. Organelle integration preserves baseline calcium cycling

Under physiological conditions and 1Hz pacing, direct integration of lysosomal and mitochondrial calcium dynamics produce minimal changes in cytosolic calcium

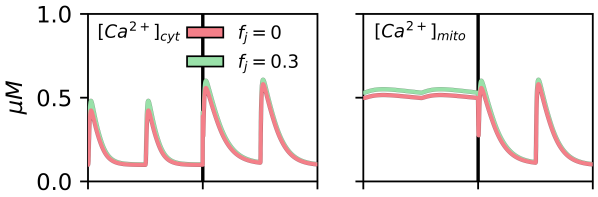

Figure 3. Cytosolic and mitochondrial calcium concentrations for different fractions of MCU channels coupled to the junctional compartment.

transients and membrane voltage in both whole-cell models (Fig. 2). Simulations under elevated extracellular calcium produced qualitatively similar responses regarding organelle interactions with baseline transients.

Sudden forced mPTP opening causes mitochondrial depolarization and increases the cytosolic calcium transient. This response aligns with enhanced ROS production, which increases RyR activity and modifies SERCA uptake.

3.2. Mitochondria-RyR proximity enhances calcium release

In T-World, mitochondria-RyR proximity is represented by the fraction of MCU channels coupled to the junctional calcium, f_j, while the remaining fraction is coupled to bulk cytosol, $f_i = 1 - f_j$. Increasing f_j to 0.3 increases the peak cytosolic calcium transient by approximately 17% relative to purely cytosolic mitochondrial coupling. (Fig. 3). This indicates that access to the junctional calcium microdomain increases the functional effect of mitochondrial calcium exchange.

Submicron simulations show a comparable dependence on mitochondria-RyR separation (Fig. 4). Reducing the minimum separation from 200 to 50 nm increases the cytosolic calcium transient by approximately 18% and moderately increases the mean junctional calcium peak during voltage-clamp stimulation. Junctional calcium is also higher in non-stimulated simulations driven by stochastic RyR activity.

In contrast to the direct junctional calcium exchange represented in T-World, the spatial response in the submicron model is mediated primarily by ROS. RyR clusters located closer to mitochondria are exposed to higher local ROS concentrations. Thus, they tend to increase RyR activity and the consequent calcium release.

4. Conclusion

We integrated lysosomal and mitochondrial calcium dynamics into est ablished human ventricular and iPSC-CM

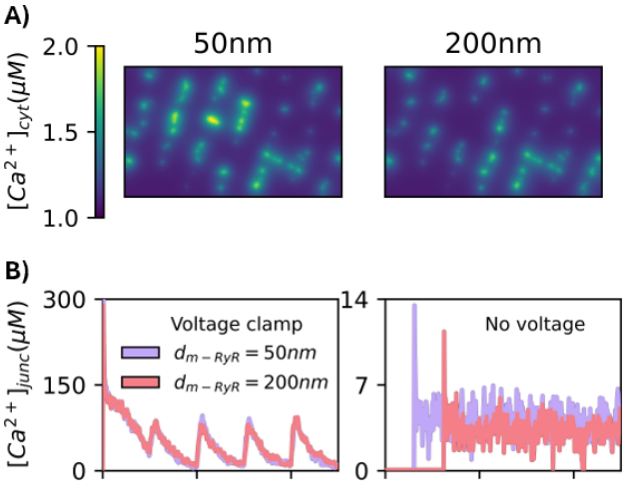

Figure 4. Effect of mitochondria-RyR separation in the submicron model. A) Cytosolic calcium snapshots for minimum separations of 50 and 200 nm at the same time frame after voltage-mediated activation. B) Mean junctional calcium during voltage-clamp stimulation and in the absence of external stimulation.

models. Under baseline conditions, organelle integration had little effect on global cytosolic calcium, aligned with the use of simpler calcium-handling formulations for simulations of physiological excitation-contraction coupling. Lysosomal calcium handling in cardiomyocytes remains poorly characterised, which has limited the development of alternative and more mechanistically detailed computational models. Although lysosomal calcium handling had no detectable effect under the conditions examined here, mitochondrial contributions became more prominent during mitochondrial depolarization and when mitochondria were located close to RyR clusters.

The whole-cell and spatial models represent mitochondria-RyR proximity through different mechanisms. In T-World, proximity allows direct coupling to junctional calcium, while in the submicron model it increases local ROS exposure. Despite this distinction, both approaches predict slightly greater calcium release with stronger mitochondrial-RyR coupling.

The models remain limited by several factors. First, the source of lysosomal and mitochondrial models are developed using data from different species and cell types and several parameters lack direct human measurements. Second, the homogeneous scaling of mitochondrial population into the whole-cell models. Third, the absence of junctional compartment in the iPSC-CM model may underestimate highly-localized organelle-RyR coupling. Fourth, the spatial geometry is also idealized and does not include reconstructed lysosomal or mitochondrial distributions.

Future work will use human iPSC-CM measurements to refine organelle-RyR coupling and investigate its contribution to calcium dysregulation, with further translation to adult human cardiomyocytes.

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