



Modeling Mitochondrial Energetics and Ion Dynamics

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During ischemia, the intracellular environment becomes more acidic and Na^+ loading occurs, affecting not only EC coupling but also the energy supplying machinery. Because ion transport determines mitochondrial pH, volume, and oxidative phosphorylation, we extended a previous single mitochondrion model to account for the dynamics of Na^+ , H^+ and phosphate exchange in addition to Ca^{2+} transport, TCA cycle flux and oxidative phosphorylation. The computational model is described by 13 ordinary differential equations, in which the kinetics of each ion carrier has been studied individually to match its behavior in *in vitro* assays. Na^+ transport through the Na^+/H^+ exchanger has been modeled as a six-state compulsory order kinetic mechanism. Phosphate transport occurs through a Pi/Ox transporter represented by a random bivalent kinetic scheme. The behavior of the assembled mitochondrial model is analyzed and compared with experiments performed with isolated mitochondria. The level of TCA cycle intermediates and the measured flux through the cycle as reported in the literature are well reproduced by the model under steady state conditions. The range of respiratory fluxes simulated corresponds to the physiological range determined in isolated mitochondria. Under transient conditions induced by pulses of ADP or protonophoric uncouplers, the model simulates a decrease in mitochondrial membrane potential ($\Delta\psi_m$), pyridine nucleotides (NADH) levels and an increase in respiratory rate. Conversely, the addition of substrate elicits reduction of the NADH pool and polarization of the membrane. This more comprehensive computational model of mitochondrial energetics and ion transport will improve our ability to study the dynamic changes in EC coupling and energetics during ischemia-reperfusion or metabolic acidosis in the future.

INTRODUCTION

A series of metabolic and ionic changes follow the onset of ischemia in cardiac ventricular myocytes. Na^+ and Ca^{2+} accumulate in the cytosol, K^+ levels decrease, lactate builds up, and the pH drops with negative consequences for the myocyte mechanical and electrical function. The mitochondria, as the main energy source providing ATP for myofibrils' contraction and ion pumping, sense and contribute to the ionic and energetic alterations. ATP production decreases and $\Delta\psi_m$ drops. In order to understand the interactions between processes involved in energy supply and demand during ischemia, an integrative effort is required, that in the present work we undertake through a combined experimental-computational modeling approach.

Herein we show results obtained with a computational model of mitochondrial energetics, pH regulation and ion dynamics, as a tool whose ultimate goal is to understand the control and regulation of energy production during ischemia-reperfusion. The model accounts for Na^+ , H^+ and PO_4 dynamics related through the Na^+/H^+ exchanger and the phosphate carrier. The model is validated through simulations that reproduce the dynamics of intermediates of the TCA cycle after a substrate challenge, or following pulses of ADP or uncouplers. This model represents a first step towards a simulation of the integrated function of the cardiac myocyte under pathological conditions.

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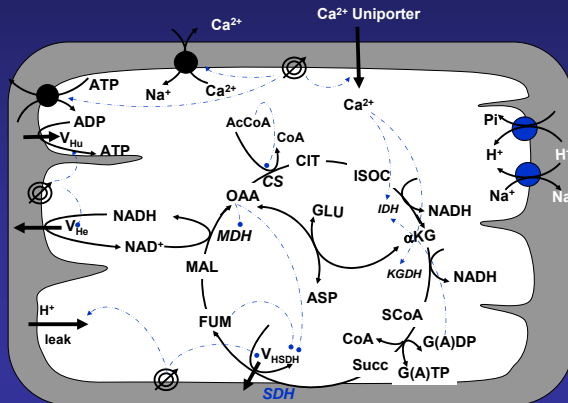


Figure 1. General scheme of the mitochondrial model.

This model is based on the Cortassa et al. model¹, which includes the TCA cycle, oxidative phosphorylation and Ca^{2+} transporters. The transporters at the inner mitochondrial membrane are adenine nucleotide translocator, $\text{Na}^+/\text{Ca}^{2+}$ antiporter, Ca^{2+} uniporter, Na^+/H^+ exchanger, and phosphate carrier. Acetyl CoA, derived from the oxidation of fatty acids and glucose, is fed into the TCA cycle. The complete oxidation of AcCoA in the TCA cycle generates NADH, that drives the electron transport through the respiratory chain. The pumping of protons across the inner mitochondrial membrane establishes an electrochemical gradient, or proton motive force ($\Delta\mu$), which in turn drives the phosphorylation of matrix ADP to ATP by the F_1F_0 -ATPase (ATP synthase).

Key to symbols: The concentric circles with an arrow across located at the inner mitochondrial membrane represent the $\Delta\psi_m$. Dotted arrows indicate regulatory interactions either positive (arrowhead) or negative (dot). Abbreviations: ASP aspartate, α KG α -ketoglutarate, CIT citric acid, FUM fumarate, IDH isocitrate dehydrogenase, ISOC isocitrate, KGDH α -ketoglutarate dehydrogenase, MAL malate, OAA oxaloacetate, SCoA succinyl-CoA, Succ succinate.

| mitochondria | EP data Randle et al. ³ | steady state (simulation) |
|----------------|---------------------------------------|------------------------------|
| unit | nM | nM |
| Citrate | 0.230 | 0.266 |
| Isocitrate | 0.030 | 0.0299 |
| 2-Oxoglutarate | 0.131 | 0.132 |
| SuccinylCoA | 0.0668 | 0.0691 |
| Succinate | 0.329 | 0.346 |
| Fumarate | 0.329 | 0.305 |
| Malate | 0.164 | 0.163 |
| Oxaloacetate | 0.0230 | 1.012E-4 |
| Sum | 1.303 | 1.3 |
| AcCoA | 0.008-0.009 | 0.001-0.01 |
| NADH+NAD | 0.86118 | 1 |

Table 1. Concentration of TCA cycle intermediates

The sum of the intermediates, AcCoA and total level of pyridine nucleotides are parameters in the model. The data in ref. 3 has been converted from mol/g dry wt into mM.

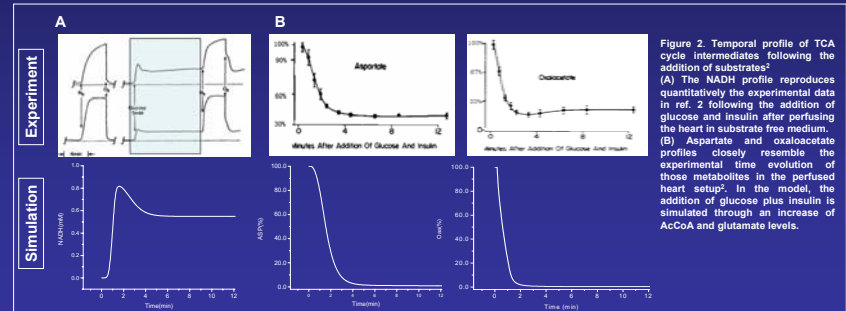


Figure 2. Temporal profile of TCA cycle intermediates following the addition of substrates. (A) The NADH profile reproduces quantitatively the experimental data in ref. 2 following the addition of glucose and insulin after perfusing the heart in substrate free medium. (B) Aspartate and oxaloacetate profiles closely resemble the experimental time evolution of these metabolites in the perfused heart setup. In the model, the addition of glucose plus insulin is simulated through an increase of AcCoA and glutamate levels.

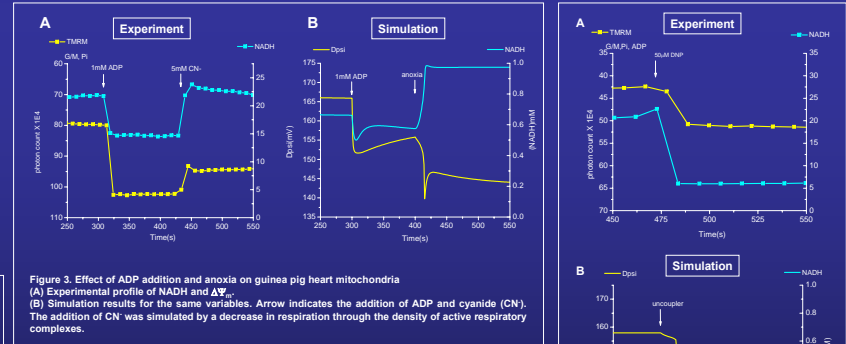


Figure 3. Effect of ADP addition and anoxia on guinea pig heart mitochondria

(A) Experimental profile of NADH and $\Delta\psi_m$. (B) Simulation results for the same variables. Arrow indicates the addition of ADP and cyanide (CN). The addition of CN was simulated by a decrease in respiration through the density of active respiratory complexes.

CONCLUSIONS

- The mitochondrial model presented is designed to study changes in energetics and ion dynamics following alterations in ionic levels such as those occurring during ischemia in cardiac myocytes.
- The mitochondria model accurately reproduces the steady state level of TCA cycle intermediates as experimentally determined in heart (3). It also reproduces experimental data of transient behavior of TCA cycle intermediates from perfused rat hearts challenged with substrate, in a semi-quantitative manner.
- The model analyzed also simulates experimental data obtained with isolated mitochondria in our laboratory concerning the temporal profile of mitochondrial NADH and membrane potential following additions of ADP, uncoupler, and anoxia.

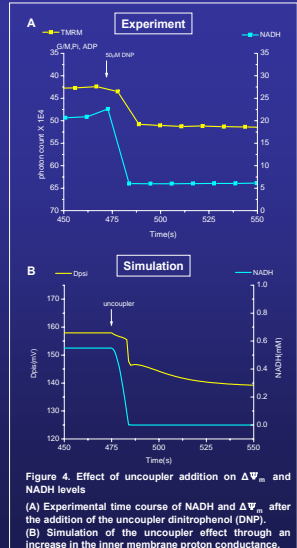


Figure 4. Effect of uncoupler addition on $\Delta\psi_m$ and NADH levels (A) Experimental time course of NADH and $\Delta\psi_m$ after the addition of the uncoupler dinitrophenol (DNP). (B) Simulation of the uncoupler effect through an increase in the inner membrane proton conductance.