

solution as well as different restrictions inside ICEUs between ATPases and mitochondria. The model is flexible and allows us to test various hypotheses regarding different compartmentalization of ATPases in the cell. This feature also makes it possible to develop the model into being able to assess a set of diffusion restrictions of more complicated systems.

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Control and Regulation of Mitochondrial Energetics in an Integrated Model of Cardiomyocyte Function

Sonia Cortassa, Brian O'Rourke, Raimond L. Winslow, Miguel A. Aon.
Johns Hopkins University, Baltimore, MD, USA.

In heart there is currently no consensus about the mechanism(s) and relative importance of the processes involved in matching energy supply with demand. This is due to mitochondrial energetics modulating and being modulated by the network of mechano-electrical processes existing in cardiomyocytes. A computational model integrating mitochondrial energetics and EC coupling provides an important analytical tool to understand the regulation and control of the global organ function. Here, we apply a generalized matrix method of control analysis to calculate flux and concentration control coefficients, as well as response coefficients, in an integrated model of Excitation-Contraction coupling and Mitochondrial Energetics in the cardiac ventricular myocyte. Control and regulation of oxygen consumption (VO_2) was first assessed in a mitochondrion model, and then in the integrated cardiac myocyte model under resting and working conditions. The results demonstrate that in the model, control of respiration is distributed among cytoplasmic ATPases and mitochondrial processes. The magnitude of control by cytoplasmic ATPases increases under working conditions. The model prediction that the respiratory chain exerts strong positive control on VO_2 (control coefficient = 0.89) was corroborated experimentally in cardiac trabeculae utilizing the inhibitor titration method. In the model, mitochondrial respiration displayed the highest response coefficients with respect to the concentration of cytoplasmic ATP (ATPi). This was due to the high elasticity of ANT flux towards ATPi. The analysis reveals the complex interdependence of sarcolemmal, cytoplasmic, and mitochondrial processes that contribute to the control of energy supply and demand in the heart. Moreover, by visualizing the structure of control of the metabolic network of the myocyte, we provide support for the emerging concept of control by diffuse loops, in which action on the network may bring about changes in processes without direct mechanistic links between them.

1241-Pos Board B85

Computational Model Of Citric Acid Cycle And Oxidative Phosphorylation In Mitochondria

Ardo Illaste, Marko Vendelin.

Tallinn University of Technology, Tallinn, Estonia.

Mitochondria are responsible for providing red muscle cells with ATP, the chemical energy of which is converted to mechanical work by sarcomeres. Just as the organism needs to cope with different levels of activity, energy production rate in mitochondria has to be able to adjust to changes in demand. We study the regulation of mitochondrial energy metabolism by ADP and inorganic phosphate with a computational model. The model consists of a thermodynamically balanced set of equations describing the reactions of the citric acid cycle, electron transfer chain and cross-membrane transport. Reactions for which enzymatic mechanisms are known are modelled accordingly. Furthermore, we account for buffering of protons by mitochondrial metabolites, yielding a system with detailed proton balance - crucial for modelling chemiosmotic energy transduction.

Suitable model parameters with which the system is able to reproduce experimental results are found from the parameter space with a combination of optimization techniques. These computationally intensive operations of solving differential equations and optimizing for parameters are automated and performed on a computational cluster.

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Application of Proportional Activation Approach to oxidative phosphorylation

Bernard Korzeniewski.

Jagiellonian University, Krakow, Poland.

Proportional Activation Approach (PAA) [1] is a simple quantitative method allowing to determine the proportional activation of the producer (P) and consumer (C) of some intermediate metabolite M by some external factor X. M can be e.g. ATP, $\Delta\Psi$ or NADH, while X can be e.g. a hormone or neural/electrical stimulation of muscle. The proportional activation of C and P ($(\Delta C/C)/(\Delta P/P)$) is quantified by the proportional activation coefficient. Application of PAA to the oxidative phosphorylation demonstrates clearly that: 1. $\Delta\Psi$ production and consumption during stimulation of isolated hepatocytes by vasopressin [1]; 2. NADH production and consumption during stimulation of isolated hepatocytes

by vasopressin [2]; 3. $\Delta\Psi$ production and consumption during electrical stimulation of rat skeletal muscle [3]; 4. ATP production and consumption during stimulation of perfused heart by adrenaline [4] - are directly activated to a similar extent. These findings confirm the so-called parallel activation idea, saying that different elements of the oxidative phosphorylation system are activated in parallel during low-to-high work transition in different cell types, that was proposed on the basis of computer simulations using a dynamic model of oxidative phosphorylation [5,6].

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1243-Pos Board B87

Cardiolipin's Structure, ATP Synthesis & Barth's Syndrome

Thomas H. Haines.

Rockefeller University, Box 187, New York, NY, USA.

Resonance stabilizes two phosphate high energy H-bonds with the free hydroxyl of cardiolipin (CL) rendering a bicyclic conformation, but only in bilayers. Thus it is symmetrical, displaying 2 pK_a 's. The pK_2 varies with the length of its fatty acid chains. With 4 $C_{18:0}$ chains the pK_2 is >8.0 . Thus the headgroup surface is a buffer at neutral pH. CL is on both sides of the IMM. The high pK_2 implies that ATP synthesis is driven by membrane potential rather than by ΔpH , lowering the energy demand for ATP synthesis. Nearly all membranes that contain CL also contain F_0F_1 , Mammalian mitochondrial CL is generally tetralinoleic, $C_{18:2}$. CL's pK_2 can be altered by chainlength and saturation. It is found on both sides of the IMM so it buffers both headgroup domains. CL binds to all 6 of the ox-phos proteins but no others in the membrane. Its high pK_2 , varies with chainlength. FAs apply a symmetrical force on the two sides of the headgroup. The bicyclic structure requires 4 chains. In lyso-CL's pK_2 is reduced to that of PG destroying the bicyclic headgroup. (our control lacks the glycerol OH). Barth's Syndrome's defective gene is a CL acyl transferase. Patients are identified by the presence of lyso-CL (3 chains).

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Quinine Causes Mitochondrial Uncoupling Independent Of K^+/H^+ Exchange Inhibition

Mohammed Aldakkak, Timothy Trichler, Amadou K.S. Camara,

Johan Haumann, David F. Stowe.

Medical College of Wisconsin, Milwaukee, WI, USA.

Introduction: K^+ influx into the respiring mitochondrial matrix is balanced by K^+ efflux via K^+/H^+ exchange (KHE). Quinine (QN) is a reversible inhibitor of KHE. We have shown that QN blocks matrix K^+ efflux when the K^+ ionophore valinomycin is given to increase matrix $[K^+]$. However QN may have other effects on mitochondria. Here we tested the effects of QN on mitochondrial respiration. **Methods:** Guinea pig heart mitochondria were isolated by differential centrifugation and then suspended in either KCl or choline Cl media inside a respirometer. Either the complex 1 substrate pyruvate (10 mM) or the complex 2 substrate succinate (10 mM) with rotenone (10 μM) was added to initiate state 2 respiration. QN (500 μM) was added to inhibit KHE. State 3 was initiated by adding ADP (250 μM) and state 4 occurred when ADP was converted to ATP. **Results:** In KCl buffer with pyruvate, QN increased states 2 and 4 respiration by $56 \pm 8\%$ and by $48 \pm 10\%$, respectively, and decreased state 3 by $15 \pm 2\%$. With succinate and rotenone, QN increased states 2 and 4 respiration by $37 \pm 3\%$ and by $15 \pm 2\%$, respectively, and decreased state 3 by $26 \pm 1\%$. QN had similar effects on respiration in choline Cl buffer. **Conclusion:** The similar effects of QN on respiration in both media suggest a K^+ -independent mechanism of QN, which also may be acting as an uncoupler to bring H^+ inside the matrix. Additional experiments show that QN lowers matrix pH without changing membrane potential. More studies with QN and other putative blockers are required to reveal the mechanism by which QN affects mitochondrial transport and bioenergetics.

1245-Pos Board B89

Mitochondrial Redox Responses To Increased Work Intensity In Rabbit Ventricular Myocytes

Iffath A. Ghouri, Ole J. Kemi, Godfrey L. Smith.

University of Glasgow, Glasgow, United Kingdom.

Simultaneous measurement of the intrinsically fluorescent metabolic coenzymes NAD(P)H (reduced) and FAD (oxidised) enabled assessment of the