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.

1240-Pos Board B84

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 (VO2) 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 VO2 (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.

1242-Pos Board B86

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 hormon 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].

[1] Korzeniewski B, Harper M-E, Brand MD (1995) Biochim Biophys Acta 1229, 315-322.
[2] Brown CG, Lakin-Thomas PL, Brand MD (1990) Eur J Biochem 192, 355-362.
[3] Rolfe DF, Newman JBM, Clark MG, Brand MD (1999) Am J Physiol 276, C692-C699.
[4] Korzeniewski B, Deschodt-Arsac V, Calmettes G, Franconi J-M, Diolez P (2008) Biochem J 413, 343-347.
[5] Korzeniewski B (1998) Biochem J 330, 1189-1195.
[6] Korzeniewski B (2006) Am J Physiol 291, H1466-H1474.

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'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 delta pH, lowering the energy demand for ATP synthesis. Nearly all membranes that contain CL also contain F_oF_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).

1244-Pos Board B88

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

mitochondrial redox response to increased work. Isolated cardiomyocytes were field stimulated and fractional shortening simultaneously recorded with epi-fluorescence measurements of NAD(P)H and FAD. Cells were paced at 0.5Hz and the stimulation frequency step increased to 1Hz, 2Hz and 3Hz in order to increase work intensity. NAD(P)H was excited at 340nm (fluorescence collected 455-480nm) and FAD was excited at 430nm (fluorescence collected 505-600nm).
Increasing the stimulation frequency from 0.5Hz to 2Hz and 3Hz, but not 1Hz, resulted in a decrease in NAD(P)H fluorescence and an increase in FAD fluorescence, indicating oxidation of the cell environment. Reducing work intensity back to 0.5Hz pacing led to immediate recovery of metabolite fluorescence. Addition of 2mM NaCN established a completely reduced mitochondrial environment, leading to NAD(P)H fluorescence increasing to a maximum and FAD fluorescence decreasing to a minimum. Subsequent step increase in stimulation to 3Hz caused no change in NAD(P)H or FAD fluorescence. Treatment with 2μM FCCP established a completely oxidised state, resulting in NAD(P)H fluorescence falling to a minimum and FAD fluorescence increasing to a maximum. Pacing at 3Hz in this state again led to no change in metabolite fluorescence, confirming the response to increased work was mitochondrial in origin. Increasing stimulation frequency to 3Hz in the presence of the movement uncoupler cytochalasin D, minimising cell contraction, also led to no change in NAD(P)H or FAD fluorescence, thus confirming that contractile work was the cause of the change in mitochondrial redox state.
In conclusion, the response to increased work intensity in cardiomyocytes is oxidation of the cell, suggesting that the mitochondria are initially unable to maintain NAD(P)H/$FADH_2$ supply in order to cope with increased metabolic demand.

1246-Pos Board B90
Trimetazidine Effects On The Mitochondrial Metabolism During Rabbit Heart Failure
Elena N. Dedkova, Lothar A. Blatter.
Rush University Medical Center, Chicago, IL, USA.
Background: We have previously shown that the anti-ischemic agent trimetazidine (TMZ) rescued $[Ca^{2+}]_i$ transient and mechanical alternans in ventricular myocytes from rabbits with non-ischemic heart failure (HF), induced by combined aortic insufficiency and stenosis. The cardioprotective action of TMZ has been linked to the inhibition of free fatty acid (FFA) oxidation, however the underlying mechanism remains poorly defined. The aim of this study was to determine whether the plasma levels of FFA (total ($[FFA]_{tot}$) and unbound to albumin ($[FFA]_u$)) are elevated in rabbit HF and whether TMZ affects mitochondrial metabolism. **Methods and Results:** We found that both $[FFA]_{tot}$ and $[FFA]_u$ were significantly elevated in HF rabbits. $[FFA]_u$ increased 4-times during HF (from 13 ± 4 to 53 ± 7 nM) while the $[FFA]_{tot}$ increased only two-fold (from 58 ± 16 to 121 ± 29 μM), demonstrating that $[FFA]_u$ is a reliable biomarker of HF. Furthermore, using TMRM fluorescence confocal microscopy and a Strathkelvin micro volume precision respirometry system, we determined that mitochondrial complex II activity was significantly elevated (+72%) during HF, while complex I activity was decreased (-90%). Cell treatment with TMZ had no effects on the complex I activity in control (+6%), while it increased (+26%) the activity of complex I under HF conditions. Moreover, TMZ reversed complex II activity in HF myocytes (-55%), while it had no effect on complex II activity in control cells (-10%). The oxidation of palmitoylcarnitine, the upstream substrate for FFA oxidation, was decreased 32% by TMZ, while TMZ had no effect on complex IV activity. Furthermore, FADH-mediated auto-fluorescence levels were significantly elevated in HF myocytes treated with TMZ. **Conclusion:** TMZ suppresses the elevated activity of mitochondrial complex II while it increases the depressed activity of complex I in rabbit HF, and therefore it preserves metabolic reserve of the cell.

1247-Pos Board B91
Mitochondrial Dynamics In Heart Cells: Very Low Amplitude High Frequency Fluctuations In Adult Cardiomyocytes And Flow Motion In Non-beating Hl-1 Cells
Nathalie Beraud[1], Sophie Pelloux[2], Yves Usson[3], Andrey V. Kuznetsov[4], Xavier Ronot[5], Yves Tourneur[2], Valdur Saks[6,7].
[1]Institute of Cybernetics, Tallinn University of Technology, Tallinn, Estonia, [2]INSERM U886 Cardioprotection et Centre de Quantimetrie, Lyon 1 University, Lyon, France, [3]CNRS UMR5525, TIMC-IMAG, Joseph Fourier University, Grenoble, France, [4]D. Swarovski Research Laboratory, Department of Transplant Surgery, Innsbruck Medical University, Innsbruck, Austria, [5]Laboratoire de Dynamique Cellulaire, UMR5525, UJF-CNRS-INPG-EPHE-ENVL, Grenoble, France, [6]INSERM U884, Laboratory of Fundamental and Applied Bioenergetics, Joseph Fourier University, Grenoble, France, [7]Laboratory of Systems Biology, Institute of Cybernetics, Tallinn, Estonia.
The arrangement and movements of mitochondria were quantitatively studied in adult rat cardiomyocytes and in the cultured continuously dividing non beating NB HL-1 cells with differentiated cardiac phenotype.
Mitochondria were stained using fluorescent dye MitoTrackerGreen, a dye associated with inner membrane of mitochondria, and studied by fluorescent confocal microscopy. Imaging during different time intervals made it possible to visualize the 2-dimensional movements and dynamics of cardiac mitochondria.
In adult cardiac cells mitochondria were always arranged very regularly in a crystal-like manner and did not show any changes in their position during 30 min of low speed scanning. However, high speed scanning (pixel dwell time 3 ms, time interval between images 400 ms) revealed very rapid fluctuations of the positions of fluorescence centers which followed the pattern of a random walk movement within the limits of the internal space of mitochondria, most probably due to transitions between condensed and orthodox configurational states of matrix and inner membrane as a result of functioning of transmembrane metabolite carriers. No evidence for mitochondrial fusion or fission was found in adult cardiomyocytes.
In contrast, in NB HL-1 cells, mitochondria were arranged as a dense tubular network, in permanent fusion, fission and displacement with high velocity around 90 nm/s.
The differences observed are related to specific structural organization of the cells, and most probably due to differences in mitochondria-cytoskeleton organization. Intracellular local restrictions of diffusion of adenine nucleotides and metabolic feedback regulation of respiration via phosphotransfer networks are also different in these cells.

1248-Pos Board B92
Cgp-37157 Abrogates The Adverse Effect Of Ouabain On Mitochondrial Energetics
Ting Liu, David A. Brown, Brian O'Rourke.
The Johns Hopkins University, Baltimore, MD, USA.
Cardiac glycosides have been used to treat heart failure for more than 200 years and their major effect is to inhibit the Na^+/K^+ pump. Inhibition of the pump on the sarcolemma of cardiac myocytes elevates intracellular Na^+ ($[Na^+]_i$), resulting in a positive inotropic effect by increasing Ca^{2+} load. However, our previous work demonstrated that elevated $[Na^+]_i$ impairs mitochondrial energetics by blunting mitochondrial Ca^{2+} ($[Ca^{2+}]_m$) accumulation. Moreover, we showed that CGP-37157, an inhibitor of $[Ca^{2+}]_m$ efflux, restored $[Ca^{2+}]_m$ accumulation and improved mitochondrial energetics. Here, we investigated the effects of ouabain with or without CGP-37157 on $[Na^+]_i$ and NADH production in isolated cardiomyocytes and examined the effects on hemodynamics and Oxygen consumption (mVO_2) in whole hearts. Application of ouabain to isolated myocytes elevated $[Na^+]_i$ in a dose-dependent way. During 1 Hz stimulation, the NADH/NAD+ redox potential in ouabain treated myocytes was decreased significantly, whereas NADH levels were well maintained in the presence of CGP-37157. In whole-heart studies, ouabain increased LVDP, +dP/dt, and -dP/dt, and addition of CGP-37157 further increased +dP/dt and -dP/dt. When isoproterenol was employed to increase cardiac work, LVDP was not increased, but +dP/dt and -dP/dt were increased by 57% and 52%, respectively, in hearts without concomitant CGP-37157 treatment. In isoproterenol-treated hearts also exposed to CGP-37157, LVDP increased by 30%, and +dP/dt and -dP/dt were increased by 75% and 53%, respectively. Whole heart mVO_2 increased by 18% after ouabain treatment and by 25% after isoproterenol administration compared to baseline. With concomitant CGP-37157 treatment, ouabain increased mVO_2 by 32% and isoproterenol increased mVO_2 by 53%. Our findings revealed an adverse effect of the glycoside on mitochondrial energetics and indicate that CGP-37157 can prevent this impairment. In addition, inotropic responses to both ouabain and isoproterenol were enhanced in the presence of CGP-37157.

1249-Pos Board B93
Mitochondrial Energetics During Transients Following Substrate And Ca2+ Additions. Modeling And Experimental Studies
An-Chi Wei[1], Miguel A. Aon[2], Brian O'Rourke[2], Raimond L. Winslow[1], Sonia Cortassa[2].
[1]Department of Biomedical Engineering, Johns Hopkins University, Baltimore, MD, USA, [2]Division of Cardiology, Johns Hopkins University, Baltimore, MD, USA.
Ionic equilibria are known to be dramatically altered in failing hearts, as well as during and after ischemic injury. Ion transport across the mitochondrial inner membrane has been shown to modulate the energetic performance of mitochondria. Consequently, it is critical to thoroughly understand the interrelationship between ion fluxes and energetics. With this aim in mind, here we continue to develop our computational model of mitochondrial energetics to account for pH regulation, Na+/H+ cotransport, and the Pi carrier, and study their effects on mitochondrial energy production and Ca2+ handling mediated by the Ca2+