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Mitochondria Dynamics in Cardiac Myocytes

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Mitochondrial dynamics are driven by the inner mitochondrial membrane potential, and have significant implications in cell function. Fission and fusion are well established characteristics of mitochondrial dynamics in most cell types. These movements are believed to be geared toward optimizing energy delivery to metabolic sites within the cell. In cardiac cells, mitochondria dynamics and their implications in cell function are poorly studied. The canonical role of mitochondria in ATP production suggests a more involved role for cardiac mitochondria dynamics into regulation of cellular function. In this study, mitochondria dynamics in adult rat, and neonatal, cardiac myocytes, are investigated. The functionality of proteins involved in the fusion and fission of mitochondria are assayed, using recently developed mitochondria targetted tools. We show that while adult cardiac myocytes exhibit relatively dormant mitochondria dynamics, mitochondria in neonatal hearts exhibit dynamics that are comparable to that of primary cell types, albeit more organized. Finally, we investigate the effects of the regulation of expression levels of mitochondrial fission and fusion proteins on calcium signaling in both cardiac cell types.

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PPARα Activation Reduced Remodelling And Improved Left Ventricular Function In Chronic Pressure/volume Overload Induced Heart Failure

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Activation of peroxisome proliferator-activated receptor-α (PPARα) in the heart is associated with a decrease of hypertrophy and fibrosis, but its overall effect on remodelling in the setting of heart failure has not been evaluated. We investigated the effect of PPARα activation by a selective and potent PPARα agonist BAYPP1: in a volume and pressure overload model of heart failure in rabbits. Volume and pressure overload was induced by induction of aortic valve insufficiency, followed by abdominal aortic banding 3 weeks later. Compound treatment started after induction of valve insufficiency. The development of HF was examined echocardiographically and electrocardiographically after 3 months. Action potentials and sodium and calcium handling were measured in left ventricular myocytes.

Rabbits with volume and pressure overload showed substantial hypertrophy and impairment of contractility compared to sham operated rabbits (table). Additionally increased lung weight, presence of ascites, increase QT duration and QRS complex and a high rate of premature death were present in HF animals. Cellular remodelling was demonstrated by: 1) increased myocyte dimensions, prolongation of the action potential, increased cytoplasmic sodium and disturbed calcium handling: a) increased diastolic calcium b) decreased amplitude and prolongation of the calcium transient c) decreased SR calcium content e) increased propensity to evoke calcium after-transient. Chronic treatment with the PPARα agonist BAYPP1 attenuated development of hypertrophy and abolished development of HF and cellular and ionic remodelling even more pronounced compared to ACE inhibition by Enalapril.

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Correlation Of Levels And Time Course Of Intracellular And Mitochondrial Calcium Signals In Isolated Adult Cardiac Cells

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The contribution of mitochondria to Ca^{2+} homeostasis in the adult cardiac myocyte is uncertain. We have successfully targeted a genetically encoded Ca^{2+} sensitive inverse pericam to the mitochondria of primary mammalian ventricular cardiomyocytes by adenoviral mediated expression (AdMitycam). Cytoplasmic Ca^{2+} ($[Ca^{2+}]_{cyt}$) and mitochondrial Ca^{2+} ($[Ca^{2+}]_{mit}$) were monitored simultaneously in adult rabbit cardiomyocytes by loading AdMityCam expressing cells with Fura2. $[Ca^{2+}]_{cyt}$ and $[Ca^{2+}]_{mit}$ were recorded during voltage clamp stimulation (0.5Hz). The sarcoplasmic reticulum (SR) was loaded at a range of $[Ca^{2+}]$ by increasing $[Ca^{2+}]_0$ (1.8-7.2mM) and by β-adrenergic stimulation (500nM isoproterenol). Beat-to-beat Ca^{2+} transients were observed in both $[Ca^{2+}]_{cyt}$ and $[Ca^{2+}]_{mit}$. The relationship between mean $[Ca^{2+}]_{cyt}$ and $[Ca^{2+}]_{mit}$ was unity at $[Ca^{2+}]$ below ~133nM, above this value $[Ca^{2+}]_{mit}>[Ca^{2+}]_{cyt}$. Under conditions of spontaneity $[Ca^{2+}]_{mit}>[Ca^{2+}]_{cyt}$ (204 ± 54.0nM vs. 118 ± 26.7nM respectively). In electrically stimulated events the kinetics of the $[Ca^{2+}]_{mit}$ transient upstroke (time-to-peak (TTP)) was significantly slower than cytoplasmic. $[Ca^{2+}]_{mit}$ TTP decreased with increasing SR Ca^{2+} load whilst $[Ca^{2+}]_{cyt}$ TTP increased. The time to 50% decay of the $[Ca^{2+}]_{mit}$ in these electrically stimulated events however was significantly *faster* than cytoplasmic. β-adrenergic stimulation further decreased the TT50% decay of the $[Ca^{2+}]_{cyt}$ by ~25% and $[Ca^{2+}]_{mit}$ by ~60%. The mechanism behind the effects of isoproterenol on the kinetics of the mitochondrial Ca^{2+} signal is unknown but may be linked to known effects of β-adrenergic stimulation on Mg^{2+} extrusion. Whilst the kinetics of the electrically triggered $[Ca^{2+}]_{cyt}$ and $[Ca^{2+}]_{mit}$ events differed, the changes in $[Ca^{2+}]_{cyt}$ and $[Ca^{2+}]_{mit}$ during spontaneous SR Ca^{2+} release events were similar in time course. These data suggest that Ca^{2+} accumulation by cardiac mitochondria depends on the time course and magnitude of the cytoplasmic Ca^{2+} signal and the presence of β-adrenergic stimulation.

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Tracking Membrane Potential, Calcium and REDOX Activity in Single Mitochondria During Normal Cardiac Cell Stimulation

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Mitochondria are well recognized as ''the powerhouse'' of the cell. In cardiac cells they may also play a role in Ca^{2+} signaling, redox signaling and cell death. They are therefore important in maintaining cardiac cellular homeostasis. By coupling fuel oxidation to ion transport across their inner membrane they create an electrochemical gradient. This electrochemical gradient is used to drive energy production to meet the cellular needs; it also drives the transport of ions, such as Ca^{2+}, across the inner mitochondrial membrane, in turn, Ca^{2+} regulates mitochondrial activity. The mitochondrial membrane potential is therefore an accurate indicator of the energetic state of the cell. The cardiac action potential continuously changes the potential of the cell membrane resulting in Ca^{2+} entry and further Ca^{2+} release from the sarcoplasmic reticulum (SR) triggering cell contraction. In freshly dissociated rat ventricular myocytes, we use confocal imaging of the fluorescent membrane potential indicator TMRE and the fluorescent Ca^{2+} indicator Rhod-2 trapped in the mitochondrial matrix to tracked changes in mitochondrial membrane potential and mitochondrial Ca^{2+} ($[Ca^{2+}]_m$) in individual mitochondria in response to electrical stimulation and caffeine induced SR Ca^{2+} release. We also tracked changes in mitochondrial NADH by measuring NADH auto fluorescence in single mitochondria under the same experimental conditions. We conclude that the intrinsic functional link between the electrical activity of the cardiac cell (due to SR Ca^{2+} release) and mitochondrial dynamics can be faithfully studied in real time by tracking mitochondrial membrane potential, Ca^{2+} or NADH in single mitochondria.

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Comparison of pH-dependence of Carbonic Anhydrase Activity i*n vitro* and in Living Cells

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Carbonic anhydrase (CA) plays an important role in acid-base homeostasis. It catalyses carbonic buffering, increases intracellular H^+-mobility and facilitates the activity of pH_i regulatory transporters. *In vitro,* CA activity is known to be inhibited by a fall of pH. This observation has been largely overlooked when considering mechanisms of pH_i regulation. There is no information, however, on the pH-sensitivity of CA in intact cells. We have therefore investigated the pH-sensitivity of CA both *in vitro,* and *in situ* in living cells. *In vitro* CA activity was assessed from the time-course of pH change after addition of CO_2-saturated water to a buffered solution (pH 8.0-6.5) containing purified bovine CA2, membrane fragments of HCT116 cells stably transfected with CA9, or cardiac homogenates from rat ventricle. *In situ* CA activity was assessed from the rate of CO_2-induced fall of pH_i in rat superfused ventricular myocytes, AM-loaded with the pH-fluorophore, carboxy-SNARF-1. The pH-sensitivity was estimated by fitting a kinetic algorithm to the pH time-course data. Results show that both *in vitro* and *in situ* CA-activity displays strong pH-dependence, activity declining with a fall of pH. Apparent pK and Hill coefficient (n_H) values for *in vitro* pH-dependence were 7.00 and 1.10 respectively for purified CA2, 6.85 & 1.73 for CA9, and 7.15 and 0.80 for cardiac homogenates. The intracellular pK_a and n_H values for generic cardiac CA were 7.20 and 2.50. These results imply that the enzyme's contribution to both intracellular and extracellular pH-regulation may vary dynamically over the physiological pH range.

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