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Dilation of human atria: Increased diffusion restrictions for ADP, overexpression of hexokinase 2 and its coupling to oxidative phosphorylation in cardiomyocytes ☆

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ABSTRACT

Cardiac energy metabolism with emphasis on mitochondria was addressed in atrial tissue from patients with overload-induced atrial dilation. Structural remodeling of dilated (D) atria manifested as intracellular accumulation of fibrillar aggregates, lipofuscin, signs of myolysis and autophagy. Despite impaired complex I dependent respiration and increased diffusion restriction for ADP, no changes regarding adenylate and creatine kinase occurred. We observed 7-fold overexpression of HK2 gene in D atria with concomitant 2-fold greater activation of mitochondrial oxygen consumption by glucose, which might represent an adaption to increased energy requirements and impaired mitochondrial function by effectively joining glycolysis and oxidative phosphorylation.

1. Introduction

Atrial dilation is a typical consequence of cardiac failure caused by hemodynamic overload (Abhayaratna et al., 2006; Burstein and Nattel, 2008; Corradi et al., 2008; Eckstein et al., 2008; Mary-Rabine et al., 1983; Schotten et al., 2003; Watson et al., 2009). Structural remodeling evidenced as myocyte hypertrophy, interstitial fibrosis, inflammatory infiltrates, impairment of gap junctions between the myocytes, reduced capillary density, as well as necrotic and apoptotic death of atrial cells has been considered to constitute a basis for atrial enlargement (Burstein and Nattel, 2008; Casaclang-Verzosa et al., 2008; Corradi et al., 2008).

Structural changes are associated with electrical and contractile dysfunction of atrial myocardium. Electrical disorders emerge as increased heterogeneity of atrial conduction, diminishing of L-type Ca^{2+} current, activation of repolarizing K^{+} currents, and decrease in intracellular Ca^{2+} transients, these changes frequently giving rise to atrial fibrillation (AF) (Casaclang-Verzosa et al., 2008; Eckstein et al., 2008; Nattel et al., 2008; Wakili et al., 2010). Diminished contractility has been related to altered Ca^{2+} handling (Allessie et al., 2002), apoptotic loss of cardiomyocytes (Aimé-Sempé et al., 1999; Moorjani et al., 2009; Thijssen et al., 2001), upregulation of fetal-like β-myosin isoform (Belus et al., 2010; Reiser et al., 2001), changes in myofilament protein phosphorylation (Belus et al., 2010), and oxidative damage of myosin heavy chain and other structural proteins (Mihm et al., 2001).

The relationships between structural, electrical and contractile remodeling are poorly understood. It has been thought that altered cellular Ca^{2+} handling underlies all three phenomena (Allessie et al., 2002; Eckstein et al., 2008; Nattel et al., 2008). However, it is also possible that energetic suppression constitutes a common ground for AF and contractile failure in diseased atria (Ashrafian et al., 2007). Indeed, changes in size, number and structure of mitochondria have been observed in cardiomyocytes in fibrillating and overloaded atria of patients or laboratory animals (Ausma et al., 1997, 2000, 2001; Laky et al., 2011; Thiedemann and Ferrans, 1977). Cha et al. (2003) detected decreased ATP content and diminished activities of creatine kinase (CK) and adenylate kinase (AK) along with dilation of dog's atria in conditions of pacing-induced congestive heart failure. Heart failure in patients with mitral regurgitation is associated with inverse relationship between CrP/ATP ratio and cardiac chamber's size (Conway et al., 1998). These changes may result from altered mitochondrial function because mechanical stretch of overloaded cardiomyocytes is a powerful inductor of mitochondria-dependent apoptosis (Liao et al., 2004; Moorjani et al., 2009).

The aim of the present study was to characterize the relationships between changes in atrial size and energy metabolism in human atrial cells, in relation to volume overload-induced atrial dilation. As a conceptual framework, it was assumed that cardiac energy metabolism is organized into the system of intracellular energy units (ICEUs). The ICEUs represent the cellular microcompartments comprising

☆ In memory of our dear friend and colleague Prof. Enn Seppet (1950–2012).

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the complexes of mitochondria and ATPases linked to each other by means of specific energy transfer and metabolic feedback signaling networks composed of CK, AK, and hexokinase (HK) isoforms functionally coupled to oxidative phosphorylation (OXPHOS) and ATPases and sharing the pool of adenine nucleotides captured within the ICEUs (Dzeja and Terzic, 2003; Dzeja et al., 2011; E.K. Seppet et al., 2005). There exists an ample evidence that the function of ICEUs is impaired in conditions of cardiac failure (Dzeja and Terzic, 2003). Recently it has been shown that if CK isoenzymes are knocked out as a hallmark of cardiovascular disease the AK- and the HK-mediated ADP transport systems increase transport capacity enabling near-normal ATP turnover (Dzeja et al., 2011). Thus, in this study, a task was set to address the changes in OXPHOS, its regulation by ADP and mitochondrial kinases in relation to the extent of atrial dilation. For several reasons, a particular attention was payed to the role of HK in remodeled atrial cardiomyocytes. It is known that an increase of mitochondrial porin bound HK activity contributes to a larger ADP supply to OXPHOS system (Aflalo, 1991; Laterveer et al., 1995) and in turn realizes a larger glycolytic flux because the product inhibition of HK by G-6-P and ADP is diminished (Laterveer et al., 1995). In muscle cells interaction between OXPHOS and glycolytic systems occurs via binding of HK type 1 or type 2 isoforms to mitochondrial voltage dependent anion channel. This interaction is promoted by ischemia or several cardioprotective agents through stimulation of PKB/Akt-mediated signaling cascade that likely serves to preserve the cellular integrity (Miyamoto et al., 2008; Southworth et al., 2007; Sun et al., 2008; Yeih et al., 2011; Zuurbier et al., 2005, 2009). It has been shown that HK stimulates OXPHOS in permeabilized HL-1 cells of cardiac phenotype (Eimre et al., 2008). At the same time, no data exists describing the interaction of HK with mitochondria in human cardiomyocytes. To fill this gap, the present work characterizes the coupling between HK and OXPHOS in human atrial myocardium, in dependence on expression of HK and extent of atrial remodeling.

2. Methods

2.1. Patients

Seventy seven patients (53 men and 24 women, average age 64 years) undergoing elective open heart surgery at the Department of Cardiac Surgery of the Tartu University Hospital participated in the study. Prior to surgery a transthoracic 2-D echocardiography was performed to assess the atrial and ventricular functions and dimensions. Right atrial (RA) minor axis dimension was measured at the end of ventricular systole. The patients having RA minor axis dimension normalized for body surface area (BSA) above normal reference ranges (Lang et al., 2006) were collected to comprise a group of subjects with dilated atria (D group). The rest of the patients were pooled to form a non-dilated (ND) group. The BSA was calculated according to Mosteller (1987).

The pieces of right atrium, detached in order to establish extracorporeal circulation during heart surgery, were used for studies. All investigations were approved by the Ethics Review Committee of the University of Tartu in accordance with the principles of the Declaration of Helsinki (WMA, 1997). All subjects signed informed consent based on their voluntary decision and agreement to undergo the procedures.

2.2. Tissue sampling and storage

Due to small size of atrial specimens obtained it was impossible to assess different parameters in the same sample. Therefore, the consecutive samples were randomly grouped for studies of different aspects of energy metabolism as indicated in legends. For enzyme analysis and determination of cytochrome content the specimens were frozen in liquid nitrogen and stored at −80 °C. For gene expression analysis the specimens were suspended in RNAlater RNA Stabilization Reagent (Qiagen, Germany). Tissue pieces meant to be used in oxygraphic or morphological studies were placed into the ice-cold buffer A containing (mM): CaK_2EGTA 1.9, K_2EGTA 8.1 (free calcium concentration 0.1 μM), $MgCl_2$ 9.5, dithiothreitol (DTT) 0.5, potassium 2-(N-morpholino)-ethanesulfonate (K-MES) 50, imidazole 20, taurine 20, Na_2ATP 2.5, phosphocreatine 15, pH 7.1 adjusted at 4 °C, immediately after removal from right atrium and kept in this medium until assessment.

2.3. Cell permeabilization and respirometry

Cardiomyocytes in atrial tissue were permeabilized by saponin in order to enable investigations of mitochondrial respiratory parameters *in situ*, in conditions of interaction of mitochondria with other intracellular structures (Kuznetsov et al., 1996). The rate of oxygen uptake of permeabilized atrial fibers was recorded by a high-resolution Oxygraph-2k (Oroboros Instruments, Austria) in solution B containing (mM): CaK_2EGTA 2.77, K_2EGTA 7.23, MgCl2 1.38, DTT 0.5, K-Mes 100, imidazole 20, taurine 20, K_2HPO_4 3, bovine serum albumin (BSA) 5 mg/ml, pH 7.1 at 25 °C. Where required, ATP or ADP was added together with $MgCl_2$ (0.8 mol/mol for ATP and 0.6 mol/mol for ADP) to keep free Mg^{2+} concentration constant in the medium.

In all experiments, the permeabilized atrial fibers were incubated in solution B at 25 °C in the presence of 10 mM glutamate and 2 mM malate for registration of the basal rate of respiration (V_0). A coupling between OXPHOS and kinases was estimated by using the following protocols:

1) 0.05 mM ATP was added to achieve a submaximal flux of endogenous ADP produced by CaMg ATPases to stimulate the respiration, 2 mM AMP was added to monitor the effect of ADP produced by mitochondrial AK2 isoform (mi-AK) and 0.2 mM diadenosine pentaphosphate (AP_5A) was supplemented to inhibit the AK activity. Capacity index of mi-AK to OXPHOS was quantified as the AK index (I_{AK}) calculated from equation: $I_{AK} = (V_{AMP} - V_{ATP})/V_{ATP}$, where V_{AMP} ja V_{ATP} are the respiration rates with AMP and ATP, respectively. Next, 20 mM creatine was added to activate mitochondrial CK (mi-CK). The efficiency of coupling of mi-CK was expressed as the CK index (I_{CK}): $I_{CK} = (V_{Cr} - V_{AP5A})/V_{AP5A}$. The experiment was continued by adding 2 mM MgADP to gain the maximum rate of OXPHOS, followed by the addition of 0.1 mM atractyloside to monitor the respiratory control via adenine nucleotide translocase (ANT).
2) Coupling of mi-CK to OXPHOS was assessed by Michaelis–Menten kinetics from relationships between [ADP] (12.5 μM to 2 mM) and corresponding rate of ADP-stimulated respiration (V_0 subtracted) in the absence and presence of 20 mM creatine.
3) To investigate the functional coupling between HK and mitochondria, after registration V_0, 2 mM ATP was added (V_{ATP}) to achieve stimulation of mitochondria with endogenous ADP produced by the ATPases. Then 10 mM of glucose was introduced (V_{gluc}) to activate HK reaction. The parallel experiments were performed in the absence and presence of 5 mM phosphoenolpyruvate (PEP) and 40 IU/ml pyruvate kinase (PK) added (V_{PK+PEP}) after registration of ATP-stimulated respiration to trap cytosolic ADP generated by ATPases. Effect of glucose was calculated as follows: $(V_{gluc} - V_{ATR})/(V_{ATP} - V_{ATR})$ or $(V_{gluc} - V_{ATR})/(V_{PK+PEP} - V_{ATR})$.
4) To assess the function of various respiratory chain complexes 2 mM ADP was added to monitor the maximum rate of NADH-linked state 3 respiration (V_{ADP}), followed by successive additions of 10 μM rotenone to inhibit complex I , 10 mM succinate to activate $FADH_2$-linked state 3 respiration (V_{Succ}), 0.1 mM atractyloside to assess respiratory control by blocking ANT (V_{Atr}), 10 μM antimycin A to inhibit the electron flow from complex II to cytochrome c (V_{Ant}),