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Stimulus specific changes of energy metabolism in hypertrophied heart

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ABSTRACT

Cardiac energy metabolism is a determinant of the response to hypertrophic stimuli. To investigate how it responds to physiological or pathological stimuli, we compared the energetic status in models of hypertrophy induced by physiological stimuli (pregnancy or treadmill running) and by pathological stimulus (spontaneously hypertensive rats, SHR) in 15 week-old female rats, leading to a 10% cardiac hypertrophy. Late stage of compensated hypertrophy was also studied in 25 week-old SHR (35% of hypertrophy). Markers of cardiac remodelling did not follow a unique pattern of expression: in trained rats, only ANF was increased; in gravid rats, calcineurin activation and BNP expression were reduced while β-MHC expression was enhanced; all markers were clearly up-regulated in 25 week-old SHR. Respiration of permeabilized fibers revealed a 17% increase in oxidative capacity in trained rats only. Mitochondrial enzyme activities, expression of the master regulator PGC-1α and mitochondrial transcription factor A, and content of mitochondrial DNA were not consistently changed, suggesting that compensated hypertrophy does not involve alterations of mitochondrial biogenesis. Mitochondrial fatty acid utilization tended to increase in trained rats and decreased by 14% in 15 week-old SHR. Expression of markers of lipid oxidation, PPARα and its down-stream targets MCAD and CPTI, was up-regulated after training and tended to decrease in gravid and 15 week-old SHR rats. Taken together these results show that there is no univocal pattern of cardiac adaptation in response to physiological or pathological hypertrophic stimuli, suggesting that other factors could play a role in determining adaptation of energy metabolism to increased workload.

1. Introduction

When the heart is submitted to a sustained increase in functional load to meet the requirement of the organism, it increases its mass as an adaptive response to normalize the pressure. Thus, hypertrophy of the myocardium usually takes place in the very early phases of cardiac stress and is accompanied by a molecular remodelling. Classically, the hypertrophic process was defined as a continuum consisting of subtle transitions that can be divided in three consecutive phases: a transitory phase, a physiological phase and lately a pathological hypertrophy where remodelling of the different compartments is no longer balanced [1]. Alternatively, cardiac hypertrophy was classified as pathological or physiological based on distinct molecular and functional cardiac adaptations [2–5].

Hypertrophy is accompanied by molecular remodelling affecting different cellular pathways. Among adaptive processes, energy metabolism plays a crucial role because it should adapt to the increased demand of the hypertrophying cardiomyocyte. Indeed, in these circumstances increased energy utilization will be required for both matching the rise in workload and allowing increased protein synthesis necessary for the hypertrophic process. It is thus possible that energy metabolism adaptations could respond differently depending on the stimulus. Cardiomyocyte hypertrophy involves an increase in intracellular organelles including mitochondria. However, while some studies showed that regular endurance exercise increases glycolysis and oxidative metabolism [6,7] others showed that mitochondrial enzyme activities parallel the increased heart weight, suggesting that adaptive responses result from a harmonious increase in muscle mass, rather than from a specific increase in mitochondrial gene expression [8,9]. How mitochondrial function and mass are regulated during hypertrophic growth and whether they can differ in response to physiological or pathological challenges require further investigation. In particular, whether adaptation to biological stresses is accompanied by changes in mitochondrial biogenesis is still poorly documented and largely controversial (see [10] for recent review). Furthermore, it is generally accepted that the hypertrophied heart shifts from lipid to glucose as substrates for energy production [11,12]. However, the generalization of the metabolic shift as an obligatory adaptive response in hypertrophy has been challenged recently [13,14] and may also differ depending on the stimuli [15]. While alterations in

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cardiac energy metabolism are considered as an important component of pathological stimulus induced-hypertrophy, probably contributing to progression to heart failure, this aspect is not elucidated following physiological stimuli. Finally, studies aimed at comparing physiological versus pathological stimuli are usually restrained to exercise training and pressure overload. Yet, many accompanying factors differ between these stimuli. For example exercise training is an intermittent challenge that generally induces a mild hypertrophy, while pressure overload is a continuous challenge that induces a larger hypertrophy. Moreover, the hormonal status also differs in response to these stimuli.

The aim of this study was thus to investigate whether the markers and metabolic phenotype of hypertrophied hearts change with the origin of the hypertrophy and as a consequence whether it could form a signature of each type of stress. Based on this concept we explored the energetic status of cardiac muscle in different female rat models with comparable degrees of hypertrophy at 15 weeks of age. Treadmill endurance training and pregnancy were used as physiological stresses while hypertension developed in spontaneously hypertensive rats (SHR), a genetic model of hypertrophy, was used as pathological stress. In SHR a later stage was also studied to assess for the consequence of prolonged pathological stimulus.

2. Materials and methods

2.1. Animals

One pathological and two physiological stresses induced cardiac hypertrophy models were used, and eight different groups of female rats were constituted. The pathological stress was hypertension in spontaneously hypertensive rats (SHR). Female 15 and 25-week-old Wistar Kyoto (15 week WKY and 25 week WKY respectively) and SHR (15 week SHR and 25 week SHR respectively) rats were obtained from Charles River (USA). Mean arterial blood pressure was measured in the two SHR groups and their respective controls at the time of sacrifice using a pressure sensor connected to a catheter introduced in the right carotid artery. Physiological stimuli were represented by physical training or pregnancy. Female 7-week-old Wistar rats, obtained from Charles River (France) were submitted to a progressive 8-week treadmill training program (2 h per day, 5 days per week, 30 m/min and 7% slope, Trained) or left sedentary (SED). 15-week-old gravid and non-gravid female Wistar rats were obtained from Charles River (France). Gravid rats were sacrificed at 18–19 days of gestation at distance from parturition to avoid massive hormonal changes.

All animals were housed 3 or 4 per cage in a temperature-controlled room (22 °C), with a 12/12 h light/dark cycle, and were provided with food and water ad libitum. At the end of experiments, all rats were anesthetized by intraperitoneal injection of pentobarbital (50 mg/kg) at between 10–11am, and the hearts were removed. Left ventricular tissue was isolated, part of which was immediately used for mitochondrial function measurement, and other part was rapidly frozen and kept at −80 °C for further investigations. All procedures were performed in accordance with the principles and guidelines established by the European Convention for the Protection of Laboratory Animals.

2.2. Study of in situ mitochondrial respiration

Oxygen consumption measurements of saponin-skinned fibers from left ventricle have been described previously [16] and exhaustively reviewed recently [17]. Different experimental protocols were used based on substrate utilization pathways as previously described [18]. The first protocol was designed to determine the sensitivity of mitochondrial respiration to fatty acids in the presence of 2 mM ADP, by addition of 4 mM malate and 0.4 mM octanoyl-carnitine. The second protocol was aimed at determining the dependency of respiration on external [ADP] and [creatine], with 10 mM glutamate and 4 mM malate as substrates [19]. Basal oxygen consumption without ADP (V_0) was recorded, and increasing amounts of ADP were added until maximal respiration was reached. The ADP-stimulated respiration (V_{ADP}) above basal oxygen consumption (V_0) was plotted as a function of [ADP] with and without creatine. The apparent K_m values for ADP and V_{ADP} were calculated with a nonlinear fit of the Michaelis–Menten equation. CK efficacy (CK_{eff}) was taken as the ratio of K_m without over K_m with creatine. The maximal respiration rate (V_{max}) was $V_{ADP} + V_0$. The coupling of phosphorylation to oxidation was determined by calculating the acceptor control ratio (ACR) as V_{max} / V_0. As no difference was observed with or without creatine for V_0, V_{max} and ACR, the data were pooled. Rates of respiration are given in μmoles O_2.min^{-1}/g dry weight^{-1} (dw). Two to three experiments were performed for each heart and each protocol.

2.3. Biochemical studies

Frozen tissue samples were weighed, homogenized (Bertin Precellys 24) in ice-cold buffer (50 mg/ml) containing (mM) 5 HEPES (pH 8.7), 1 EGTA, 1 DTT and 0.1% Triton X-100. Citrate synthase (CS), cytochrome *c* oxidase (COX), complex I of the mitochondrial respiratory chain (CxI) activities were measured at 30 °C (pH 7.5) by spectrophotometry as previously described [20].

2.4. Real-time quantitative PCR analysis

Total cardiac RNA was extracted using standard procedures. Oligo-dT first strand cDNA was synthesized from 5 μg total RNA using superscript II reverse transcriptase (Invitrogen). Total cellular DNA was extracted from left ventricle by standard methods including successive steps of proteinase K digestion, organic extraction and ethanol precipitation [21] to quantify mitochondrial DNA using specific primers for 12SrRNA. Real-time PCR was performed using the SYBR®-Green method on a LightCycler rapid thermal cycler (Roche Diagnostics) as previously described [21]. PCR amplification was performed in a total reaction volume of 15 μl. The reaction mixture consisted of 5 μl diluted template, 1.5 μl FastStart DNA Master SYBR Green I kit (×10), 3 mM $MgCl_2$ (except for the slow myosin heavy chain isoform (β-MHC) and 12SrRNA with 3.5 mM, and brain natriuretic peptide (BNP) with 5 mM) and 0.5 μM forward and reverse primers (Table 1). After initial denaturation at 95° for 8 min and activation of Taq polymerase, amplification was allowed to proceed for 30–40 cycles, each consisting of denaturation at +95 °C for 10 s, annealing at specific temperature (Table 1) for 5 s and extension at +72 °C for 5–25 s, depending on the length of the PCR product (25 bp per second). Fivefold serial dilution from cardiac total cDNA or DNA (for 12SrRNA) was analyzed for each target gene and allowed us to construct linear standard curves from which the concentration of the test sample was calculated. For cDNA amplification, forward and reverse primers were designed in a different exon of the target gene sequence, eliminating the possibility of amplifying genomic DNA. A basic local alignment search (BLAST) performed for each set of primers revealed that sequence homology was obtained only for the target gene. Glucocerebrosidase (GCB) was chosen as housekeeping gene for normalization of the mRNA amount as its expression did not differ between control and experimental groups. Results were first normalized to GCB transcription in order to compensate for variation in input RNA amounts and efficiency of reverse transcription, then they were multiplied by total RNA (or DNA for 12SrRNA) per amount of tissue (μg/mg wet weight^{-1}) to compare expression levels in different conditions [21].

2.5. Statistical analysis

Data are expressed as mean ± SEM. Statistical significance was defined as $p<0.05$ with the non parametric *U* Mann–Whitney test to