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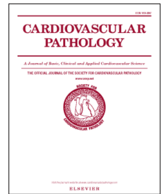

Original Article

Compromised mitochondrial remodeling in compensatory hypertrophied myocardium of spontaneously hypertensive rat

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ABSTRACT

Background: Hypertension leads to cardiac hypertrophy as an adaptive response to increased workload. While initial development of hypertrophy is compensatory when contractile function is maintained, persistent stress on heart leads to deteriorated cardiac function and onset of heart failure. Mitochondrial dysfunction develops in the failing heart; however, whether it presents in compensatory cardiac hypertrophy is controversial.
Methods: Spontaneously hypertensive rats (SHRs) and age-matched normotensive Wistar Kyoto rats were used in the study. Mitochondrial function and remodeling-related mechanisms in the left ventricles were measured by enzyme activity tests, Western blots, and reverse transcriptase polymerase chain reaction.
Results: Compensatory cardiac hypertrophy in SHR was indicated by higher heart/weigh ratio, left ventricular systolic pressure and $\pm dp/dt_{max}$ ($P<.001$, $P<.05$, and $P<.01$, respectively). Enzyme activities of mitochondrial complex I and II were significantly reduced ($P<.05$ and $P<.01$) in SHR in concert with decreased expression of complex subunits ($P<.01$ for NDUFS3, $P=.068$ for SDHB, and $P<.05$ for ATP5A1). Mitochondrial fission protein Drp1 was decreased ($P<.05$), while fusion protein OPA1 was increased ($P<.01$). Parkin and SirT1/AMPK-PGC-1α signaling, responsible for mitochondrial elimination and biogenesis respectively, were decreased in SHR ($P<.01$ for Parkin, $P<.001$ for SirT1 and p-AMPK).
Conclusion: Our results implicated that mitochondrial function and remodeling, indicated by mitochondrial enzyme activities and remodeling-related molecules, were compromised in compensatory hypertrophied myocardium of the SHR hypertensive model.
Summary: Mitochondrial function in compensatory hypertrophied myocardium is controversial. Our present study found mitochondrial dysfunction in the left ventricle of spontaneously hypertensive rats, which was possibly a result of compromised mitochondrial remodeling including mitochondrial dynamics, elimination, and biogenesis.

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1. Introduction

Hypertension is a significant health menace afflicting over 1 billion people over the world [1]. Increased pressure overload during hypertension progression leads to changes in cardiac structure particularly in the left ventricle (LV), producing left ventricular hypertrophy [2]. Initially, the development of hypertrophy is a compensatory mechanism, and contractile function is maintained [3]. However, when the heart is excessively and/or persistently stressed, cardiac function may deteriorate, leading to the onset of heart failure [3].

Mitochondria are the main source of energy in eukaryotic cells and are abundant in high-energy-demanded organs like the heart. Mitochondrial quality is precisely controlled by mitochondrial remodeling mechanisms including biogenesis and repair, dynamics, and mitophagy [4]. Disturbance in any of the mechanisms results in mitochondrial dysfunction, which is widely seen in diseases. Considering the close relationship between workload and energy generation demand, cardiac hypertrophy will inevitably leads to alterations in mitochondrial function. Recent investigations suggest mitochondrial dysfunction to be tightly associated with the development of cardiac hypertrophy as well as hypertension [5]. It is now generally accepted that mitochondrial dysfunction develops in the

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failing heart. However, whether mitochondrial dysfunction presents in compensatory cardiac hypertrophy is of great controversy. By using surgical or no-surgical experimental models of cardiac hypertrophy, some studies reported unchanged mitochondrial function or morphology [6], while others suggested mitochondrial function to be disorganized by observing disturbed mitochondrial metabolism, decreased enzyme activity, or oxidative stress [7–9] at compensatory hypertrophied myocardium.

Spontaneously hypertensive rats (SHRs) are a commonly employed experimental model of hypertension and cardiac hypertrophy [10]. In the present study, we aim to determine mitochondrial function in the hypertrophied LVs of SHR animals and investigate remodeling-related molecules as potential mechanisms that regulate mitochondrial function.

2. Materials and methods

2.1. Animal study

The SHR is a well-established model of genetic hypertension. SHR is prehypertension for the first 6–8 weeks and develops hypertension over the next 12–14 weeks [10]. Cardiac pump function is preserved at 1 year of age, while heart failure develops at 18–24 months in SHR [11].

Twenty-four-week-old male SHR (exhibiting both apparent hypertension and compensatory cardiac hypertrophy [8]) and age-matched male Wistar Kyoto (WKY) rats were housed in separate cages in a temperature-controlled room (22–24°C) under a 12-h light/12-h dark cycle with free access to food and water. All animal procedures described in this study were performed in adherence with the *Principle of Laboratory Animal Care* published by the US National Institutes of Health (NIH publication No. 86-23, revised 1985), with approval from the Fourth Military Medical University Committee on Animal Care.

For assessment of heart rate and cardiac hemodynamic parameters, rats were anaesthetized with sodium pentobarbital (30 mg/kg, intravenously). A polyethylene catheter was inserted into the LV through the left atrial appendage. LV pressure was continually recorded via a data acquisition system (PowerLab 300 system, ID, Sydney, Australia). The heart rate, left ventricular end-diastolic pressure (LVEDP), left ventricular systolic pressure (LVSP), maximal rate of pressure rise ($+dp/dt_{max}$) and fall ($-dp/dt_{max}$) were automatically analyzed.

The whole hearts were excised, weighed, and wash thoroughly with cold saline to remove contamination blood. The LV was separated and placed immediately in liquid nitrogen and stored at −80°C until use.

2.2. Measurement of mitochondrial enzyme activities

Mitochondria were isolated as previously described [12] and used for assay of mitochondrial enzyme activities. Measurement of NADH-CoQ oxidoreductase (complex I), succinate-CoQ oxidoreductase (complex II), CoQ-cytochrome *c* reductase (complex III), cytochrome *c* oxidase (complex IV), and ATP synthetase (complex V) activities were performed as previously described [13].

2.3. Detection of ATP content

Snap-frozen cardiac samples were homogenized in adequate ice-cold lysis buffer (100 mM glycine, 1% Triton X-100, pH 7.4) and centrifuged at 15,000*g* for 10 min at 4°C. Supernatants were assayed for ATP assay bioluminescent assay kit (Sigma Chemical Co., St. Louis, MO, USA) according to the manufacturer's protocol and bioluminescence was recorded. Values were normalized to protein concentration of each sample.

2.4. Western blotting

For protein immunoblot assay, LV was homogenized and protein concentrations were determined using BCA protein assay kit (Thermo Scientific, Rockford, IL, USA). Soluble lysates (10 μg per lane) were subjected to dodecyl sulfate polyacrylamide gel electrophoresis. Proteins were then transferred to nitrocellulose membranes and blocked. Membranes were incubated with primary antibodies for OxPhos Complexes I 30-kDa subunit, Complex II 30-kDa subunit, Complex Vα (Invitrogen, Carlsbad, CA, USA), GAPDH, VDAC1, SirT1, PGC1, mtTFA, P62, Mfn 1, Mfn 2 (Santa Cruz, Heidelberg, Germany), AMPK, phospho-AMPKα (Thr172), Atg5, Atg7, Beclin1 and Parkin (Cell Signaling Technology, Beverly, MA, USA), Drp1, OPA1 (BD Biosciences, San Joes, CA, USA), and horseradish peroxidase-conjugated secondary antibodies (Jackson ImmunoResearch, West Grove, PA, USA). Western blots were developed using Pierce ECL Western blotting substrate (Thermo Scientific) and quantified by scanning densitometry.

2.5. Real-time polymerase chain reaction

For real-time polymerase chain reaction (PCR) expression analysis, total RNA was extracted using Trizol reagent (Invitrogen) and reverse transcribed using the PrimeScript RT reagents kit (TaKaRa Biotechnology, Dalian, China), according to the manufacturer's protocol. Synthesized complementary DNA was amplified using specific primers (purchased from AuGCT DNA-SYNBiotechnology, Beijing, China) for rat *Nrf1* [14] (forward, 5′-TTACAGGGCGGTGAAATGAC-3′; reverse, 5′-GTTAAGGGCCATGGTGACAG-3′); rat *ND1* (forward, 5′-TTCGCCCTATTCTTCATAGCCG-3′; reverse, 5′-GGAGGTGCATTAGTTGGTCATATCG-3′); and rat GAPDH [9] (forward, 5′-AGACAGCCGCATCTTCTTGT-3′; reverse, 5′-CTTGCCGTGGGTAGAGTCAT-3′). Quantitative PCR was performed with SYBR-Green Master Mix (TaKaRa Biotechnology) according to the manufacturer's protocol using a real-time PCR system (Eppendorf, Hamburg, Germany). Relative gene expression levels were adjusted as the values relative to GAPDH. The WKY group was used as the calibrator with a given value of 1, and SHR group was compared with this calibrator.

2.6. Statistics

All values are expressed as means±SEM. Unpaired *t* test was performed to evaluate differences between groups using SPSS Statistics V17.0 software. A *P* value less than .05 was considered statistically significant.

3. Results

3.1. Heart hypertrophy and cardiac hemodynamic parameters in SHR

The 24-week-old SHR had apparently lower body weight than their control WKY, while their heart weights were mildly increased, leading to a significantly increased ratio of heart to body weight (Table 1).

Table 1
Animal characteristics

	Body weight (g)	Heart weight (mg)	Heart/Body weight (mg/g)
WKY(n=8)	447.9±15.4	1223.8±36.8	2.74±0.07
SHR(n=8)	367.4±5.1***	1271.3±85.3	3.46±0.08***

Values are means±SEM.
*** P<.001.

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