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Original Article

ATPase may play a critical role in disturbance of energy metabolism in congestive heart failure rats

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Abstract: Objective: This study aims to explore the expression and significance of ATPase in myocardium of congestive heart failure induced by pressure overload in rats. Methods: Male SD rats were divided into 3 groups randomly: Control group (C group), coarctation of abdominal aorta 15 weeks group (A group) and sham operation group (SH group). The hemodynamics parameters were measured. The size of adenine acid pool (ATP, ADP) in myocardium was measured by high performance liquid chromatography (HPLC). The mRNA copies of ATPase-α subunit, ATPase-6 subunit in ventricular myocardium were determined by RT-PCR. The protein content of F_1-ATPase in mitochondria was detected by Western blot analysis. Results: The $\pm dp/dt_{max}$ and contents of ATP and ADP decreased significantly in myocardium were decreased in A group. Compared with C and SH group, the mRNA copies of ATPase-α subunit, ATPase -6 subunit and the protein content of F_1-ATPase were decreased in A group. Conclusion: The expression of F_1-ATPase decreased parallel with abnormal of the adenine nucleotide in myocardium of congestive heart failure. It may play a critical role in disturbance of energy metabolism in disfunction of heart.

Keywords: Congestive heart failure, ATPase, mitochondria

Introduction

Cardiovascular disease is one of the leading causes of death and disability in the world. Although there has been a decrease in mortality of acute myocardial infarction over the last years, there has been a concomitant rise in mortality attributable to heart failure (HF). The syndrome of HF was described by Hippocrates over two millennia ago and presented as shortness of breath and peripheral edema [1, 2]. The heart is a high energy consuming organ and has an extraordinarily high capacity for ATP resynthesis. Reduced capacity for ATP production is inextricably associated with the severity of heart failure. The phosphocreatine (PCr)/ATP ratio which reflects the mitochondrial energy reserve state was confirmed to be reduced in previous studies [3-6]. Furthermore, the ratio of PCr to ATP was considered to be a better predictor of cardiovascular mortality in failing heart subjected to energy metabolism alterations [7]. At the end stages of HF, the myocardium has low ATP content due to a decreased ability to generate ATP by oxidative metabolism, which is unable to effectively transfer the chemical energy from the metabolism of carbon fuels to myocardial cellular contractile work [8].

The mechanism of metabolic dysfunction in HF is poorly understood. Mitochondria are the main places to produce energy in cells. The complex V-adenosine three phosphate synthase (ATPase) in its endometrium is the key enzyme for energy synthesis and a large number of synthesized ATP supply the myocardium cells to maintain normal physiological activities. The expression of ATPase and changes of myocardial adenylate content in myocardium of congestive heart failure induced by pressure overload in rats was determined in this study to explore the mechanism of metabolic dysfunction in HF.

Table 1. Primers of ATPase-α subunit, ATPase-6 subunit and β-actin

Gene	5'-3'
β-actin	TTGTCACCAACTGGGACGATATGG
	GATCTTGATCTTCATGGTGCTAGG
ATPase-6	AGCCAGGGCCGGAAGTAGAAC
	ACGCCAGTCGCTTTGCTTTGT
ATPase-α	CAGAACAGTCGGATGCAAAG
	TCCACAAGTCCCAGGATACA

Methods

Experimental animals

A total of 39 11-14 week-old male healthy Sprague-Dawley rats were obtained from Experimental Animal Center of Fifth Hospital of PLA. They were divided into 3 groups randomly: coarctation of abdominal aorta group (A group), sham operation group (SH group) and control group (C group). Each group has 13 rats. Laparotomy was carried out after anesthesia by intraperitoneal injection of 3% pentobarbital sodium. Abdominal artery was stripped at approximately 5 mm from above the left renal artery opening, a 6/0 silk suture was tied around and made up to 65%~70% constriction of abdominal aorta. Sham operated animals underwent the same procedure except the ligation. They were treated with anti-infection for 3 days and observed for 15 weeks. Housing and procedures involving experimental animals were in accordance with the Guide for the Care and Use of Laboratory Animals (eighth edition, published by the National Academies Press). All animal experiments were approved by the Animal Care and Studies committee of the Fifth Hospital of PLA.

Detection of hemodynamic parameters

Hemodynamic parameters were detected at 15 weeks after operation. Rats were anesthetized and right carotid artery intubation was carried out. The pressure curves of aorta and left ventricle were recorded with physiological recorder. Left ventricular end-diastolic pressure (LVEDP), mean aortic pressure (MAP) and rate of rise/descend of left ventricular pressure ($\pm dp/dt_{max}$) were measured.

Isolation of myocardial mitochondria

Myocardial mitochondria were isolated using a high-speed centrifugation by sucrose gradient method modified from the protocol described by Mariuaz [9]. Mitochondria were isolated at 4°C and mitochondrial protein concentration was determined by Lowry's method.

Determination of adenine acid concentrations in myocardial tissue

The adenine acid concentrations in myocardial tissue were determined using the method described by Schonfeld [10]. The fresh myocardial tissue block (100 mg) was homogenized in pre-cooling 0.4 M perchloric acid ($HClO_4$) for measuring ADP and ATP concentrations. 20 ul of supernatant was assayed by high performance liquid chromatography (HPLC).

RNA purification and quantitative RT-PCR

Total RNA of myocardial tissue was isolated using RNeasy kits (QIAGEN, Germantown, MD). One step of reverse quantitative PCR was performed using iScript One Step RT-PCR Kit with SYBR Green (Bio-Rad, USA). Primer sequences of ATPase-α subunit, ATPase-6 subunit and β-actin were listed in **Table 1**. Candidate gene expression of ATPase-α subunit, ATPase-6 subunit was normalized to β-actin expression.

Expression of F_1-ATPase protein in myocardial mitochondria

Tissues were lysed in RIPA buffer containing 50 mM Tris (pH 7.4), 150 mM NaCl, 1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS, 1 mM EDTA and protease inhibitor cocktail (Beyotime, China) for proteins extracts. Mitochondrial protein and total proteins were separated by sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gel and transferred to a polyvinylidene difluoride membrane. Membrane was incubated with goat anti F_1-ATPase polyclonal antibody (1:400) and a-tubulin (1:5000). The secondary antibodies were conjugated at the dilution of 1:1000 and bands were stained with DAB. Bands were analyzed with quantity one image analysis software (Bio-Rad, USA).

Statistical analysis

Values are presented with mean ± SD. All data analysis was performed with SPSS 17.0 software. The data were analyzed with one-way ANOVA and correlation analysis. LSD test was used in multiple comparison. $P<0.05$ was considered to be statistical significance.

Table 2. Hemodynamical changes in rats (Mean ± SD)

Group	MAP (mmHg)	LVEDP (mmHg)	+dp/dt$_{max}$ (mmHg/s)	-dp/dt$_{max}$ (mmHg/s)
C	78.50 ± 6.70	3.84 ± 0.35	9780.23 ± 560.81	7851.50 ± 320.72
SH	76.59 ± 3.55	4.02 ± 0.25	9977.19 ± 43.45	7758.27 ± 304.56
A	124.46 ± 3.94**	6.38 ± 0.60**	8660.07 ± 237.27**	6023.46 ± 892.08**

**: p<0.01. A group: coarctation of abdominal aorta 15 week group; SH group: sham operation group; C group: control group; LVEDP: Left ventricular end-diastolic pressure; MAP: mean aortic pressure; ±dp/dt$_{max}$: rate of rise/descend of left ventricular pressure.

Table 3. Changes of adenine acid concentrations in myocardium in rats (Mean ± SD)

Group	ATP (nmol/mg)	ADP (nmol/mg)	AMP (nmol/mg)
C	1.54 ± 0.30	2.34 ± 0.65	2.81 ± 0.78
SH	1.46 ± 0.30	2.36 ± 0.70	2.89 ± 0.80
A	0.52 ± 0.28**	0.73 ± 0.49**	1.21 ± 0.64**

**: p<0.01. A group: coarctation of abdominal aorta 15 week group; SH group: sham operation group; C group: control group.

Table 4. The mRNA expression of ATPase subunits in myocardium in rats (Mean ± SD)

Group	ATPase-α/β-actin	ATPase-6/β-actin
C	8.45 ± 2.18	10.64 ± 3.07
SH	9.03 ± 2.34	11.00 ± 3.93
A	4.58 ± 1.88**	6.13 ± 1.87*

*: p<0.05; **: p<0.01. A group: coarctation of abdominal Caorta 15 week group; SH group: sham operation group; C group: control group.

Results

Hemodynamical changes in rats

The results of hemodynamical changes showed that the MAP, LVEDP, +dp/dt$_{max}$ and -dp/dt$_{max}$ of A group were 124.46 ± 3.94, 6.38 ± 0.60, 8660.07 ± 237.27 and 6023.46 ± 892.08 respectively. Compared with SH group, the MAP and LVEDP increased and ±dp/dt$_{max}$ decreased significantly in A group (P<0.01). There was no significant difference between SH group and C group in MAP, LVEDP and ±dp/dt$_{max}$ (P>0.05). Data were shown in **Table 2**.

Adenine acid concentrations in rats

As shown in **Table 3**, there was no significant difference between SH group and C group in adenine acid concentrations (P>0.05). The concentrations of ATP, ADP and AMP were 0.52 ± 0.28, 0.73 ± 0.49 and 1.21 ± 0.64 respectively. Compared with SH group, the concentrations of ATP, ADP and AMP decreased significantly in A group (P<0.01).

Expression of ATPase subunits in myocardium in rats

The results of ATPase subunits expression showed that the ATPase-α/β-actin of C group, SH group and A group were 8.45 ± 2.18, 9.03 ± 2.34 and 4.58 ± 1.88 respectively. The ATPase-6/β-actin of C group, SH group and A group were 10.64 ± 3.07, 11.00 ± 3.93 and 6.13 ± 1.87 respectively. There was no significant difference between SH group and C group (P>0.05). Compared with SH group, the expression of ATPase-α subunit and ATPase-6 subunit decreased significantly in A group (P<0.01 and P<0.05 respectively, **Table 4**).

Expression of F_1-ATPase in myocardium in rats

The western blotting results of F_1-ATPase expression were shown in **Figure 1**. The F_1-ATPase/α-tubulin of C group, SH group and A group were 0.98 ± 0.29, 0.97 ± 0.23 and 0.44 ± 0.13 respectively. There was no significant difference between SH group and C group (P>0.05). Compared with SH group, the expression of F_1-ATPase decreased significantly in A group (P<0.01, **Table 5**).

Discussion

Many myocardial cellular physiology functions were mainly on the base of energy metabolism. ATP is main high-energy phosphate carrier in cells which has 3 phosphates. ADP and AMP were generated after the phosphate was split and transferred usually. ATP is directly used for cardiac muscle fiber contraction. The results of this study showed that left ventricular dysfunction, increased MAP and LVEDP, decreased ±dp/dt$_{max}$ presented in 15-week pressure over-

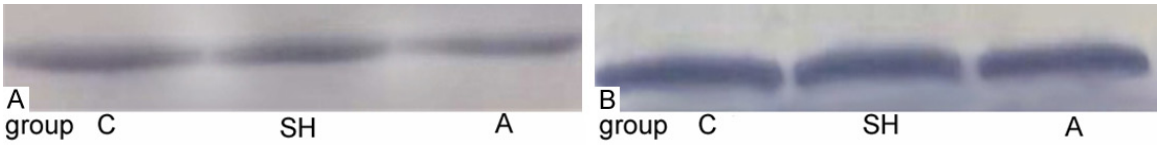

Figure 1. Western blotting results of F_1-ATPase and α-tubulin. A: Western blotting results of F_1-ATPase; B: Western blotting results of α-tubulin. A group: coarctation of abdominal aorta 15 week group; SH group: sham operation group; C group: control group.

Table 5. The expression of F_1-ATPase in myocardium in rats (Mean ± SD)

Group	F_1-ATPase/α-tubulin
C	0.98 ± 0.29
SH	0.97 ± 0.23
A	0.44 ± 0.13**

**: p<0.01. A group: coarctation of abdominal aorta 15 week group; SH group: sham operation group; C group: control group.

load rats. At the same time, the ATP, ADP and AMP concentration decreased in myocardial tissue. These results suggested that myocardial energy metabolism dysfunction occurred in HF and high-energy phosphate significantly decreased, which was consistent with previous studies [11, 12].

ATPase is a large transmembrane complex which including many subunits. It is composed three parts: the head (F_1), base (F_0) and a handle part. F_1 is composed of α3, β3, γ, δ and ε polypeptides encoded by nuclear gene (nDNA) and nine different subunits, which is the catalytic core of ATPase [13]. F_0 is embedded in the inner mitochondrial membrane containing 11 different subunits, of which ATPase subunit 6 and subunit 8 are encoded by mtDNA. F_0 is the main component of proton channel, which can transfer protons across the membrane to the catalytic site of F_1.

Expression of the ATPase subunit plays an important role in the enzyme activities [14, 15]. Studies have found that up-regulated and down regulated ATPase biosynthesis are involved in transcriptional and post-transcriptional regulation. Abnormal ATPase biosynthesis can produce two types of defects. One is the qualitative change caused by the change of enzyme structure and the other is quantitative change caused by the lack of enzyme content. The two changes weakened the utilization of proton gradient and made obstacles to the cell energy supply [16]. Shertzer et al. [17] found that ATPase deficiency is the main reason for the decrease of ATP levels in tissues and the increase of respiratory rate. Hu et al. [18] examined whether decreases of high-energy phosphates and mitochondrial F (1) F (0)-ATPase subunits typical of failing myocardium exist in border zone myocardium of compensated post-infarct remodeled hearts. They found that energetic insufficiency in the peri-infarct region may contribute to the transition from compensated LV remodeling to congestive heart failure. Liu et al. [19] found that the mitochondrial protein levels of the F (0) F (1)-ATPase subunits were normal in hearts with compensated LV remodeling. However, in HF, the α-subunit decreased by 36%, the β-subunit decreased by 16%, in HF, reductions in mitochondrial F (0) F (1)-ATPase protein expression are associated with increased myocardial free ADP.

In conclusion, we found that the expression of ATPase decreased in HF was related to the declined catalytic ability of enzyme and abnormalities of high-energy phosphate. It suggested that decreased expression of ATPase in mitochondria induced deficiency mitochondrial energy synthesis, which involved in metabolism abnormity of myocardial high-energy phosphate, eventually leading to myocardial cell dysfunction.

Disclosure of conflict of interest

None.

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