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Original Article

Apelin administration ameliorates high fat diet-induced cardiac hypertrophy and contractile dysfunction

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ABSTRACT

Apelin has been recognized as an adipokine that plays an important role in regulating energy metabolism and is credited with antiobesity and antidiabetic properties. This study was designed to examine the effect of exogenous apelin on obesity-associated cardiac dysfunction. Oral glucose tolerance test, echocardiography, cardiomyocyte contractile and intracellular Ca^{2+} properties were assessed in adult C57BL/6 J mice fed – low or a – high-fat diet for 24 weeks followed by apelin treatment (100 nmol/kg, i.p. for 2 weeks). High-fat diet resulted in increased left ventricular diastolic and systolic diameters, and wall thickness, compromised fractional shortening, impaired cardiomyocyte mechanics (peak-shortening, maximal velocity of shortening/relengthening, and duration of shortening and relengthening) and compromised intracellular Ca^{2+} handling, all of which were reconciled by apelin. Apelin treatment also reversed high fat diet-induced changes in intracellular Ca^{2+} regulatory proteins, ER stress, and autophagy. In addition, microRNAs (miR) -133a, miR-208 and miR-1 which were elevated following high-fat feeding were attenuated by apelin treatment. In cultured cardiomyocytes apelin reconciled palmitic acid-induced cardiomyocyte contractile anomalies. Collectively, these data depict a pivotal role of apelin in obesity-associated cardiac contractile dysfunction, suggesting a therapeutic potential of apelin in the management of cardiac dysfunction associated with obesity.

1. Introduction

Obesity is increasing in prevalence and is recognized as an independent risk factor for heart failure. Uncorrected obesity has been shown to lead to dyslipidemia, hypertension, insulin resistance, all of which may predispose devastating cardiac complications such as cardiac hypertrophy [1–3]. Although several mechanisms have been postulated to identify obesity-induced cardiac dysfunction, neither precise mechanism nor effective therapeutic remedy is available for obesity-induced cardiac remodeling and contractile dysfunction.

Adipokines such as leptin and adiponectin, secreted by adipose tissue play a pivotal role in the development of obesity and insulin resistance [4,5]. Apelin is a recently discovered adipokine which has been credited with regulating a variety of biological functions [6–10]. Recent studies have shown that apelin improves insulin sensitivity in obese, insulin resistant mice, whereas knockout of apelin leads to glucose intolerance [11–13]. Lower levels of apelin have been implicated in cardiovascular disease (reviewed in [14]. However, the possible beneficial effect of apelin in obesity-induced cardiac remodeling and contractile dysfunction is largely unknown. Because downregulation of apelin and apelin receptors (APJ) correlate to a decline in cardiac performance, it is plausible to speculate that apelin administration improves cardiac function [14]. Therefore, the present study was designed to determine whether apelin attenuates cardiac hypertrophy and cardiac contractile dysfunction associated with diet-induced obesity. Because ER stress and autophagy have been implicated in obesity-associated cardiac remodeling and contractile dysfunction, essential protein markers of ER stress and autophagy were monitored. As certain muscle specific microRNA especially miR-1, miR-133a and miR-208 serve as unique regulators for cardiac hypertrophy and contractile function, microRNA levels were assessed in low fat and high fat-fed mice treated with or without apelin [15,16].

2. Methods

2.1. Experimental animals and diet

All animal procedures described here were in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85–23, revised 1996) and was approved by the University of Wyoming Animal Care and Use Committee. In brief, 4-week-old male C57BL/6 J mice were randomly assigned to low-fat or high-fat for 6 months. The diet used for these studies were commercially available from Research Diets (New Brunswick, NJ). Catalog # D12450B with 10 kcal% fat

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was used as the control diet and Cat# D12492 was used as the high fat (60 kcal% fat) diet. Lard was the source of lipid in the high-fat diet. In separate experiments, mice subjected to a high-fat diet (as described above) were subsequently placed on a low-fat diet until they achieved weight reductions that were observed with apelin treatment, and cardiac parameters were evaluated by echocardiography as described below. Following confirmation of insulin resistance using glucose tolerance test, mice were treated with either apelin-13 fluoroacetate (100 nmol/kg/day, i.p.) or vehicle (saline) for 2-weeks while maintained on their respective low or high-fat diet.

2.2. Oral glucose tolerance test (OGTT)

OGTT was performed at the beginning and the end of the treatment period following a 12-hour fasting period [17]. In brief, mice were fasted for 12 h and were challenged with glucose (2 g/kg, intraperitoneally). Blood samples were drawn from the tail vein immediately before glucose challenge, as well as 30, 60, 90, and 120 min thereafter. Blood glucose levels were determined using a glucometer.

2.3. Insulin, triglyceride, cholesterol, and leptin

Serum levels of triglycerides and total cholesterol were measured using commercial kits (from Biovision, Milpitas, CA) per manufacturer's protocol. Leptin and Insulin were measured using commercial kits (from Millipore, Billerica, MA) per manufacturer's protocol.

2.4. Echocardiography

Cardiac geometry and function were evaluated in anesthetized (ketamine 80 mg/kg and xylazine 12 mg/kg, ip) mice using a two-dimensional (2D) guided M-mode echocardiography (Phillips Sonos 5500) equipped with a 15–6 MHz linear transducer (Phillips Medical Systems, Andover, MD, USA). The adequate depth of anaesthesia was monitored using the toe reflex. The heart was imaged in the 2D mode in the parasternal long-axis view with a depth setting of 3 cm. An M-mode cursor was positioned perpendicular to interventricular septum and posterior wall of the left ventricular (LV) at the level of the papillary muscles from the 2D mode. Diastolic wall thickness, end diastolic dimension (EDD) and end systolic dimension (ESD) were measured. All measurements were done in accordance with the Guidelines of the American Society of Echocardiography [18]. The percentage of LV fractional shortening was calculated as [(EDD-ESD)/EDD] × 100 [19].

2.5. Blood pressure measurement

Mouse systolic and diastolic blood pressure was measured by a CODA semi-automated non-invasive blood pressure device (Kent Scientific Co, Torrington, CT) [17].

2.6. Histology and oil red staining

Ventricular tissues were stained with FITC-conjugated wheat germ agglutinin and cardiomyocyte cross-sectional area was quantitated by measuring 150 random cardiomyocytes. For lipid staining hearts were embedded in Tissue-Tek Optimal Cutting Temperature compound (Sakura) and ventricular sections were stained with oil red O (details in the supplementary methods section).

2.7. Cardiomyocyte isolation and mechanics

Mouse cardiomyocytes were isolated using liberase enzymatic digestion; mechanical properties were assessed using an IonOptix soft-edge system (Ionoptix, Milton, MA) as described previously (see supplementary methods for details) [20]. Cell shortening and relengthening were assessed using peak shortening (PS), time to PS, time to 90% relengthening, and maximal velocities of shortening/relengthening (dL/dt). To assess the effect of apelin on saturated fatty acid-induced cardiomyocyte dysfunction, murine cardiomyocytes were incubated with palmitic acid (PA, 75 μM) [20] for 2 h in the presence or absence of apelin (500nM) prior to mechanical assessment. Palmitate-containing media were prepared by conjugation of palmitic acid with fatty acid-free bovine serum albumin (BSA), by a method described previously [21]. Given that palmitic acid was dissolved in a BSA-containing solution, BSA (150 μM) was included as a negative control for cardiomyocyte experiment.

2.8. Intracellular Ca^{2+} transients

Separate cohorts of myocytes were loaded with fura-2/AM (0.5 μM) for 15 min, and fluorescence intensity was measured with a dual-excitation fluorescence photomultiplier system (IonOptix). Myocytes were placed on an inverted Olympus microscope and imaged through a Fluor 40x-oil objective as described previously [22].

2.9. Western blot analysis

Protein expression of key Ca^{2+} regulatory proteins (SERCA2a, phospholamban (PLB)], autophagy markers (beclin-1, LC3B, and Atg7), ER stress markers (Bip and CHOP), and hypertrophy markers (calcineurin A, GATA 4, NFATC3 and GSK3β) were examined using Western blot analysis. Details of the antibodies used are listed in Table S1.

2.10. miRNA extraction and quantitative real-time PCR analysis

Total RNA containing miRNA was extracted using a *mir*Vana™ miR isolation kit (Ambion, Inc. Austin, TX) and was used for the quantitative real-time PCR (qRT-PCR) analysis. 20 ng of total RNA containing miR was reverse-transcribed individually with a *mir*Vana™ qRT-PCR miR-detection kit (Applied Biosystems, Foster City, CA) using the specific reverse transcribed primers for miR-1, miR-133a and miR-208. Then the cDNA from miRs were used for the PCR assay based on the instruction of the manufacturer, specific primers for miR-1, miR-133a and miR-208 were used. Relative gene expression was normalized by U6 gene expression.

2.11. Mitochondrial respiration and fatty acid oxidation capacity

Mitochondrial respiration was examined in freshly isolated mitochondria (75 μg protein) using the two-channel high resolution respirometer (Oroboros Oxygraph; Innsbruck, Austria) in the presence of 100 μM ADP and saturating concentrations of (in mM): pyruvate (5), malate (5), glutamate (10) and succinate (10) to fully reconstitute supply of reducing equivalents from TCA cycle enzymes to respiratory complexes 1 and 2. State 3 (ADP-stimulated) and state 4 (ADP-limited; leak) respiration was determined, as well as the respiratory control ratio (State 3/4) and ADP/O ratio (oxygen consumed during state 3 respiration) to provide indices of the phosphorylation control and efficiency of mitochondrial respiration. To determine the effects of diet and apelin on mitochondrial fatty acid beta-oxidation activity, we assessed the enzymatic activity of β-hydroxyacyl-CoA dehydrogenase (BHAD), which catalyzes the third and rate-limiting step of the beta-oxidation pathway, generating NADH for the mitochondrial respiratory system. See Supplemental Methods for detailed experimental procedures.

2.12. Caspase-3 assay and cell viability

Caspase-3 activity was determined colorimetrically using the Caspase-3 assay kit according the manufacturer's instructions. Cell viability was assessed by eh MTT assay based on the transformation