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mTOR-Independent autophagy inducer trehalose rescues against insulin resistance-induced myocardial contractile anomalies: Role of p38 MAPK and Foxo1

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ABSTRACT

Insulin resistance is associated with cardiovascular diseases although the precise mechanisms remain elusive. Akt2, a critical member of the Akt family, plays an essential role in insulin signaling. This study was designed to examine the effect of trehalose, an mTOR-independent autophagy inducer, on myocardial function in an Akt2 knockout-induced insulin resistance model. Adult WT and Akt2 knockout ($Akt2^{-/-}$) mice were administered trehalose (1 mg/g/day, i.p.) for two days and were then given 2% trehalose in drinking water for two more months. Echocardiographic and myocardial mechanics, intracellular Ca^{2+} properties, glucose tolerance, and autophagy were assessed. Apoptosis and ER stress were evaluated using TUNEL staining, Caspase 3 assay and Western blot. Autophagy and autophagy flux were examined with a focus on p38 mitogen activated protein kinase (MAPK), Forkhead box O (Foxo1) and Akt. Akt2 ablation impaired glucose tolerance, myocardial geometry and function accompanied with pronounced apoptosis, ER stress and dampened autophagy, the effects of which were ameliorated by trehalose treatment. Inhibition of lysosomal activity using bafilomycin A1 negated trehalose–induced induction of autophagy (LC3B-II and p62). Moreover, phosphorylation of p38 MAPK and Foxo1 were upregulated in $Akt2^{-/-}$ mice, the effect of which was attenuated by trehalose. Phosphorylation of Akt was suppressed in $Akt2^{-/-}$ mice and was unaffected by trehalose. In vitro findings revealed that the p38 MAPK activator anisomycin and the Foxo1 inhibitor (through phosphorylation) AS1842856 effectively masked trehalose-offered beneficial cardiomyocyte contractile response against Akt2 ablation. These data suggest that trehalose may rescue against insulin resistance-induced myocardial contractile defect and apoptosis, via autophagy associated with dephosphorylation of p38 MAPK and Foxo1 without affecting phosphorylation of Akt.

1. Introduction

Insulin resistance, or impaired sensitivity to insulin, is a hallmark of metabolic morbidities including type 2 diabetes mellitus, obesity and hypertension, clustered together as the "metabolic syndrome" [1,2]. Incidence of cardiovascular disease is much higher in individuals with insulin resistance, as evidenced by dampened cardiac energy utilization efficiency (glucose oxidation versus fatty acid oxidation), compromised ventricular function and coronary heart diseases [1]. Insulin resistance-associated myocardial anomalies are reminiscent of those found in diabetic individuals with compromised heart function, characterized by impaired ventricular compliance, prolonged action potential duration and ventricular diastole, and delayed cytosolic Ca^{2+} clearance [3–5]. It is suggested that compromised insulin signaling in the heart plays an important role in insulin resistance-induced myocardial contractile and geometric anomalies [6,7]. Within the complex insulin regulatory signaling cascade, the phosphatidylinositol 3-kinase (PI3K)-Akt pathway governs the metabolic properties of insulin and serves as an essential gate-keeper for post-insulin receptor signaling transduction [8,9]. Akt, a serine/threonine kinase downstream of PI3K, comprises three isoforms Akt1, Akt2 and Akt3. Although the structure and function of these three isoforms are highly conserved, ample studies have depicted somewhat distinct yet overlapping roles for these isoforms in physiological and pathophysiological

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conditions [10,11]. Dysregulation of Akt is known to trigger a number of diseases including cardiovascular diseases, metabolic disorders and cancer [12–14]. A dominant negative Akt2 mutation (R274H) has been demonstrated to be linked with severe hyperinsulinemia and diabetes mellitus in human [15]. In mouse models, knockout of Akt2 triggers overt global pre-diabetic insulin resistance [11,16] and myocardial contractile dysfunction [17] although the precise mechanism(s) underscoring Akt2 ablation-associated myocardial contractile and geometric derangements remains elusive.

Autophagy is a highly conserved intracellular lysosomal catabolic process to degrade aged, damaged or dysfunctional proteins, intracellular organelles and cytoplasmic components to maintain cellular homeostasis [18–20]. Basal level of autophagy plays a unique housekeeping role in the regulation of cardiac geometry and function [21–23]. Impaired autophagy may contribute to various end organ complications in insulin resistance and diabetes, including cardiomyopathy and nephropathy [24,25]. Autophagy is believed to be downregulated in insulin resistance while tissue-specific autophagy knockout mice displayed overt insulin resistance [25–27]. Autophagy is usually regulated by both mTOR (mammalian target of rapamycin)-dependent and –independent mechanisms [28]. mTOR pathway is deemed the classical regulation route of autophagy, which negatively regulates autophagy involving two functional complexes: mTORC1 and mTORC2, with a much more predominant role for mTORC1. Inhibition of mTORC1 can induce autophagy which is associated with reduced phosphorylation of two downstream targets p70S6K (ribosomal protein S6 kinase-1) and 4E-BP1 (translation initiation factor 4E-binding protein-1) [18,29]. Various signaling molecules/pathways may function as upstream regulators for mTORC1 to control autophagy, including Rag (Ras-related GTP-binding protein) [30,31], PI3K/Akt/TSC (tuberous sclerosis complex) [29,32,33] and AMPK/TSC [34–37]. Despite the regulation of autophagy by mTORC1 and its upstream signaling components, several mTOR-independent pathways may also participate in the regulation of autophagy. Up-to-date, a number of cellular machineries including inositol [38], Ca^{2+}/calpain [39], cAMP/Epac/Ins [1,4,5] r3 [39], c-Jun N terminal kinase (JNK)/Beclin1/PI3 K [40], and p38/Atg9 [41] have been reported to regulate autophagy in a mTOR-independent manner.

Trehalose, a natural occurring α-linked disaccharide widely distributed in non-mammalian species such as fungi, yeast, bacteria, invertebrates, insects and plants, functions to provide energy sources and protects the integrity of cells against various environmental stresses [42–45]. Trehalose has also been demonstrated to protect against apoptosis in an autophagy-dependent manner [42,43]. Trehalose induces autophagy by facilitating the recruitment of LC3B to the autophagosomal membranes in an mTOR-independent manner [42]. Nonetheless, the precise signaling mechanism underneath trehalose-regulated autophagy still remains unclear. To this end, this study was designed to examine the effect of Akt2 knockout-induced insulin resistance on myocardial function and geometry as well as the impact of trehalose on Akt2 knockout-induced myocardial anomalies, if any. To better elucidate the underlying mechanism of trehalose-induced autophagy in the regulation of cardiac geometry and function, potential mTOR-independent cellular regulatory pathways were examined. Our data suggested that Akt2 deletion may directly contribute to the down-regulation of autophagy and up-regulation of apoptosis and ER stress due to the loss of transmission of insulin signaling to mTORC1 pathway. More importantly, trehalose treatment was capable of restoring the level of autophagy (likely through improved autophagy flux) and inhibiting apoptosis in a p38 MAPK-dependent albeit mTOR-independent manner.

2. Materials and methods

2.1. Experimental animals and trehalose treatment

All animal experimental procedures carried out here were approved by the Animal Use and Care Committees at the University of Wyoming (Laramie, WY, USA). Adult Akt2 knockout ($Akt2^{-/-}$) mice were obtained from Prof. Morris Birnbaum at the University of Pennsylvania (Philadelphia, PA, USA) and their wild-type (WT) littermates were used as wild-type controls [11]. All mice were housed in a temperature-controlled room (22.8 ± 2.0 °C, 45–50% humidity) under a 12 h/12 h light/dark and allowed access to food and water ad libitum. WT and Akt2 knockout mice were divided into two groups: one group with trehalose treatment, mice were first administreated with trehalose (1 mg/g/day) intraperitoneal injection for two days and then given 2% (w/v) trehalose (Sigma-Aldrich Ltd., St. Louis, MO, USA) in drinking water for two months, while the other group were offered regular drinking water [46].

2.2. Intraperitoneal glucose tolerance teat (IPGTT)

Following trehalose treatment, mice were fasted for 12 h and were then given an intraperitoneal injection of glucose (2 g/kg body weight). Blood samples were drawn from tail veins and serum glucose levels were determined immediately before glucose challenge, as well as 15, 30, 60 and 120 min thereafter using the CONTOURTM NEXT EZ blood glucose monitoring system (Bayer Diabetes Care, Tarrytown, NY, USA) [47].

2.3. Echocardiographic assessment

Cardiac geometry and function were evaluated in anesthetized (ketamine 80 mg/kg and xylazine 12 mg/kg, i.p.) mice using the two-dimensional guided M-mode echocardiography (Philips SONOS 5500) equipped with a 15–6 MHz linear transducer (Phillips Medical Systems, Andover, MD, USA). Left ventricular (LV) anterior and posterior wall dimensions during diastole and systole were recorded from three consecutive cycles in M-mode using the method adopted by the American Society of Echocardiography. Fractional shortening was calculated from LV end-diastolic (LVEDD) and end-systolic (LVESD) diameters using the equation (LVEDD − LVESD)/LVEDD × 100. Echocardiographic LV mass was estimated using the following equation [(LVEDD + septal wall thickness + posterior wall thickness)^3 − LVEDD^3] × 1.055, where 1.055 (mg/mm3) is the density of myocardium. Heart rates were averaged over 10 consecutive cycles [37,48].

2.4. Isolation of murine cardiomyocytes

After ketamine/xylazine sedation, hearts were removed and were mounted onto a temperature-controlled (37 °C) Langendorff system. Following perfusing with a modified Tyrode solution (Ca^{2+} free) for 2 min, the heart was digested for 16–20 min with a Ca^{2+}-free KHB buffer containing Liberase Blendzyme 4 (Hoffmann-La Roche Inc., Indianapolis, IN, USA). The modified Tyrode's solution (pH 7.4) contained (in mM): NaCl 135, KCl 4.0, $MgCl_2$ 1.0, HEPES 10, NaH_2PO_4 0.33, glucose 10, and butanedione monoxime 10, and the solution was gassed with 5% CO_2–95% O_2. Digested hearts were then removed from the cannula and left ventricles were cut into small pieces in the modified Tyrode's solution. Tissue pieces were gently agitated and pellet of cells was resuspended. Extracellular Ca^{2+} was added incrementally back to 1.20 mM over a period of 30 min. A yield of at least 60–70% viable rod-shaped cardiomyocytes with clear sarcomere striations was achieved (which was unaffected by Akt2 ablation or trehalose treatment). Only