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Carbonylation of myosin heavy chains in rat heart during diabetes

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ABSTRACT

Cardiac inotropy progressively declines during diabetes mellitus. To date, the molecular mechanisms underlying this defect remain incompletely characterized. This study tests the hypothesis that ventricular myosin heavy chains (MHC) undergo carbonylation by reactive carbonyl species (RCS) during diabetes and these modifications contribute to the inotropic decline. Male Sprague–Dawley rats were injected with streptozotocin (STZ). Fourteen days later the animals were divided into two groups: one group was treated with the RCS blocker aminoguanidine for 6 weeks, while the other group received no treatment. After 8 weeks of diabetes, cardiac ejection fraction, fractional shortening, left ventricular pressure development (+d*P*/d*t*) and myocyte shortening were decreased by 9%, 16%, 34% and 18%, respectively. Ca^{2+}- and Mg^{2+}-actomyosin ATPase activities and peak actomyosin syneresis were also reduced by 35%, 28%, and 72%. MHC-α to MHC-β ratio was 12:88. Mass spectrometry and Western blots revealed the presence of carbonyl adducts on MHC-α and MHC-β. Aminoguanidine treatment did not alter MHC composition, but it blunted formation of carbonyl adducts and decreases in actomyosin Ca^{2+}-sensitive ATPase activity, syneresis, myocyte shortening, cardiac ejection fraction, fractional shortening and +d*P*/d*t* induced by diabetes. From these new data it can be concluded that in addition to isozyme switching, modification of MHC by RCS also contributes to the inotropic decline seen during diabetes.

1. Introduction

Approximately 250 million people worldwide have diabetes mellitus (DM) and this number is expected to reach near 400 million by 2030 [1,2]. The situation is equally disturbing in the United States where about 9% of the population has DM [3]. Even more worrying is the three to five fold higher rates of cardiovascular diseases, including heart failure in these individuals in coming decades. Diabetic cardiomyopathy (DC) starts as an asymptomatic slowing in cardiac relaxation kinetics [4]. As the syndrome progresses, inotropy, fraction shortening and ejection fraction decline leading to an increase in morbidity and mortality [5]. To date, the etiology underlying these cardiac contractile defects remains incompletely characterized.

Myosin heavy chain (MHC) is the motor protein responsible for force generation. It is part of the myosin complex that is made up of six polypeptides: two MHC (≈220 kDa) isozymes intertwined at their tails with theirs heads separated and containing one essential light chain (≈18 kDa) and one regulatory light chain (≈22 kDa) [6]. The myosin complex is divided into three functionally distinct regions: a motor and a lever arm domain that are located in the globular head and a tail or rod domain. The motor domain contains the ATP binding pocket and the actin-binding site [7,8]. The lever arm consists of two IQ motifs that form attachment sites for the essential and regulatory light chains. The tail region forms the thick filaments of muscle sarcomere. Following depolarization, Ca^{2+} released from the internal sarcoplasmic reticulum (SR) bind to troponin C that resides on actin filaments. Ca^{2+} binding to troponin C alters its conformation and increases its affinity for troponin I [9]. The increased troponin C-troponin I interaction creates an opening that allows MHC to bind and form weak cross-bridges with actin. The binding of MHC to actin exposes its nucleotide-binding pocket, allowing ATP to bind. Following ATP binding, a narrow cleft between the upper and lower domains of the motor head on MHC

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opens, reducing MHC affinity for actin. Immediately after detaching, hydrolysis of ATP occurs resulting in the formation of a metastable ternary complex between myosin, ADP and inorganic phosphate (P_i). The release of P_i reinitiates strong binding of the motor head of MHC to actin. Elastic bending and interaction of the essential light chains with actin induces the power stroke (force generation). During the power stroke, conformational changes induced by ATP are reversed, the nucleotide-binding pocket reopens, ADP is released and the rigor conformation is reestablished to restart the process [8,10]. Thus, a series of well-timed MHC conformational changes are needed to ensure effective contraction.

Two isoforms of MHC are expressed in mammalian heart: MHC-α and MHC-β. In healthy adult rodents, MHC-α is the dominant isozyme. Following stressors like diabetes, MHC isozymes switch such that MHC-β becomes the dominant heart isozyme [11,12]. This switching starts as early as 1 week after the induction of DM as a result of reductions in production and circulation of thyroid hormones [13,14]. Since the ATPase activity of MHC-β is two-to-three fold less than that of MHC-α, studies attribute the reduction in inotropy seen during diabetes, in part to MHC isozyme switching [11–14]. However, in animals models of diabetes including the widely used streptozotocin-induced type 1 diabetic rat model, measurable decline in cardiac inotropy typically starts after 2–3 weeks of diabetes and progresses as the duration of diabetes increases [15,16]. Moreover, intensive lowering of blood glucose does not prevent the inotropic decline in patients with diabetes mellitus [17,18]. This and other data prompted us to investigate whether factors other than isozyme switching may be further reducing MHC activities and cross-bridge kinetics during diabetes.

Shortly after the onset of hyperglycemia, production of reactive carbonyl species (RCS) increases as a result of enhanced glucose and fatty acid oxidation and increased expression of RCS generating enzyme serum semicarbazide amine oxidases [19,20]. These electrophiles can react with the exposed amine/azide group of basic residues on proteins to form carbonyl adducts [21] by a process referred to as carbonylation. For proteins like MHC that have slow a turn over rate of >5 days [22], adducts will accumulate over time. More than 20 years ago, Yudkin et al. [23] found elevated levels of glucose adducts (glycosylation) on MHC from post-mortem hearts of diabetic patients. Others have since confirmed these findings [24–26]. What remains incompletely defined to date is whether other types of carbonyl adducts are formed on MHC during DM and whether the presence of these adducts compromise the rate at which MHC hydrolyzes ATP to generate the power stroke. Thus, the objectives of present study were three fold: (i) to assess whether other carbonyl adducts are formed on MHC during DM, (ii) to determine which amino acid residues on MHC susceptible to carbonylation, and (iii) to determine if carbonyl adducts formed on MHC during diabetes impair ATP hydrolyze and cross-bridge formation with actin.

2. Materials and methods

2.1. Chemicals and drugs

Ketamine (Ketaset®) was obtained from Fort Dodge Animal Health, Fort Dodge, IA, USA, acepromazine from Boehringer Ingelheim Vetmedica Inc., (St. Joseph, MO). Aminoguanidine bicarbonate, Na_2-ATP and Inactin® were obtained from Sigma–Aldrich (St. Louis, MO). mAb F59 and mAb S58 monoclonal antibodies used to detect total MHC (MHC-α and MHC-β and MHC were obtained from The Developmental Studies Hydridoma Bank, University of Iowa, Iowa City, IA. Actin (C-11), anti-goat, and anti-mouse IgG-horseradish peroxidase were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Collagenase type 2 was obtained from Worthington Biochemical Corp., Lakewood, NJ. Serum T3 was measured in triplicate by radioimmunoassay kits (Diagnostic Products Corporation, Los Angeles, CA), total thiobarbituric acid reactive substances (TBARS) were assayed using OXI-TEK (TBARS) assay kits (Zepto Metric Corporation, Buffalo, NY) and serum semicarbazide-sensitive amine oxidase was assayed using SSAO assay kits (Cell Technology, Inc., Mountain View, CA). Insulin pellets were obtained from LinShin Canada Inc., Scarborough, CANADA. Standard reagents and buffers used were of the highest grade available and also purchased from Sigma–Aldrich (St. Louis, MO).

2.2. Induction and verification of experimental type 1 diabetes mellitus

Animals used for the study were approved by the Institutional Animal Care and Use Committee, University of Nebraska Medical Center and adhered to APS's Guiding Principles in the Care and Used of Animals [27]. Eighty-four male Sprague–Dawley rats (≈200 g) were purchased from Sasco Breeding laboratories (Omaha, NE). Laboratory chow and tap water were given *ad libitum*. After acclimatization for 1 week, 50 rats were given a single intraperitoneal (i.p.) injection (0.25 mL) with freshly prepared streptozotocin (STZ) in a 2% solution of cold 0.1 M citrate buffer, pH 4.5 (45–50 mg/kg) as described earlier [28]. The other 34 rats were injected with a similar volume of citrate buffer only. Throughout the experimental protocol, blood glucose levels of diabetic animals were maintained between 19.2–25 mmol (350–450 mg/dL) by inserting 0.5 mm × 2 mm insulin pellets subcutaneously. To generate euglycemic diabetic controls, 0.5 mm × 5 mm insulin pellets were inserted after 6 weeks of diabetes. The latter is referred to as the insulin therapy group. Animals were housed in pairs of similar weights to minimize dominance at 22 °C with fixed 12 h light/12 h dark cycles and 30–40% relative humidity.

2.3. Treatment to reduce reactive carbonyl species (RCS)

Two weeks after STZ injection, diabetic rats were randomly divided into three groups. One group of was placed on aminoguanidine (Ag) treatment (1.0 g/L/day, 0.1%) via drinking water for 6 weeks [29]. A second group was treated with insulin after 6 weeks of diabetes to attain euglycemia and the third group remained untreated. Control animals were also divided into two groups: one group was placed on Ag treatment at a higher dose of 1.5 g/L for 6 weeks while the other remained as untreated. The higher dose of Ag was used for treating control rats as we earlier found that they drink about 66% less water per day than diabetic rats (150 mL/day *vs* 400 mL/day). Ag was selected for this study because of the multiple mode by which it inhibits RCS.

2.4. Assessment of cardiac function in vivo

2.4.1. M-mode echocardiography

M-mode echocardiography was performed at the end of the 8-week protocol in lightly anesthetized (0.3 mL of a cocktail containing 100 mg/ml ketamine and 10 mg/mL acepromazine given i.p.) animals using an Acuson Sequoia 512C ultrasound system (Siemens) with a 15L8 probe as described earlier [28]. Left ventricular end-diastolic diameter (LVEDD), end-systolic diameter (LVESD), left ventricular end-diastolic volume (LVEDV) and end-systolic volume (LVESV) were measured parameters. Percent fractional shortening (FS) was calculated as FS = [(LVEDD − LVESD)/LVEDD] × 100. Percent ejection fraction (EF) was also calculated as EF = [(LVEDV − LVESV)/LVEDV] × 100.