Archives of Biochemistry and Biophysics 558 (2014) 1–9

Contents lists available at ScienceDirect

Archives of Biochemistry and Biophysics

journal homepage: www.elsevier.com/locate/yabbi

Expression and induction of small heat shock proteins in rat heart under chronic hyperglycemic conditions

V. Sudhakar Reddy, Ch. Uday Kumar, G. Raghu, G. Bhanuprakash Reddy *

Biochemistry Division, National Institute of Nutrition, Hyderabad, India

ARTICLE INFO

Article history:
Received 13 February 2014
and in revised form 2 June 2014
Available online 17 June 2014

Keywords:
sHsp
Diabetes
Diabetic cardiomyopathy
Streptozotocin
Phosphorylation
p38MAPK
p44/42MAPK
Apoptosis
Rat

ABSTRACT

The induction of small heat shock proteins (sHsp) is observed under various stress conditions to protect the cells and organisms from adverse events including diabetes. Diabetic cardiomyopathy is a common complication of diabetes. Therefore, in this study, we investigated the expression of sHsp under chronic hyperglycemic conditions in rat heart. Hyperglycemia was induced in WNIN rats by intraperitoneal injection of streptozotocin and maintained for a period of 12 weeks. Expression of sHsp, phosphorylation and translocation of phosphoforms of Hsp27 and αB-crystallin (αBC) from cytosolic fraction to cytoskeletal fraction was analyzed. While the expression of MKBP, HspB3, αBC was found to be increased in diabetic heart, expression of Hsp20 was decreased. Chronic hyperglycemia further induced phosphorylation of αBC at S59, S45, Hsp27 at S82, p38MAPK and p44/42MAPK. However, pS59-αBC and pS82-Hsp27 were translocated from detergent-soluble to detergent-insoluble fraction under hyperglycemic conditions. Furthermore, the interaction of pS82-Hsp27 and pS59-αBC with desmin was increased under hyperglycemia. However, the interaction of αBC and pS59-αBC with Bax was impaired by chronic hyperglycemia. These results suggest up regulation of sHsp (MKBP, HspB3 and αBC), phosphorylation and translocation of Hsp27 and αBC to striated sarcomeres and impaired interaction of αBC and pS59-αBC with Bax under chronic hyperglycemia.

Introduction

Diabetes has become a serious public health problem. The number of diabetic patients was 382 million in 2013 and is expected to reach 592 million in 2030 [1]. Diabetic cardiomyopathy is a common complication of diabetes mellitus and is one of the most common causes of morbidity and mortality in diabetic patients. Diabetic cardiomyopathy is a major risk factor for developing myocardial dysfunction in diabetic patients in the absence of hypertension and coronary heart disease [2–5]. Diabetes is associated with disturbed myofibrils, severe alterations in sarcomere microstructure and components of dystrophin associated protein complex [6]. However, the pathophysiological insults for the development of diabetic cardiomyopathy are poorly understood. Though the cause of diabetic cardiomyopathy is not fully understood, oxidative stress, cardiac inflammation, lipid accumulation, cardiac fibrosis and apoptosis are considered to be the major mechanisms implicated in diabetic cardiomyopathy [2,7]. Of these, oxidative stress induced by excessive production of reactive oxygen species (ROS) and reactive nitrogen species (RNS)[1] resulting from hyperglycemia causes cardiac fibrosis [8], altered signaling pathways, altered gene expressions [9] and myocardial cell death [10,11].

Heat shock proteins (Hsp) are a group of proteins that accumulate in the cells after a variety of physiological, environmental and pathological stresses. Small Hsp (sHsp) are proteins with monomeric molecular mass ranging from 15 to 30 kDa and with a conserved α-crystallin domain. Mammals contain 10 sHsp: Hsp27/HSPB1, myotonic dystrophy kinase binding protein (MKBP)/HSPB2, HSPB3, αA-crystallin (αAC)/HSPB4, αB-crystallin (αBC)/HSPB5, Hsp20/HSPB6, cvHsp/HSPB7, Hsp22/H11/H2IG1/HSPB8, HSPB9 and sperm outer dense fiber protein (ODF)/HSPB10 [12]. Small Hsp acts as molecular chaperones by preventing aggregation or misfolding of proteins and allow their correct refolding under stress conditions [13–15]. These proteins are also involved in several fundamental cellular processes like cytoskeletal architecture, intracellular transport of proteins and protection against programmed cell death [15]. The heat shock response is mediated by a group of heat shock transcription factors (HSF). The mammals

* Corresponding author. Address: National Institute of Nutrition, Hyderabad 500 007, India. Fax: +91 40 27019074.
E-mail address: geereddy@yahoo.com (G.B. Reddy).

[1] *Abbreviations used:* ROS, reactive oxygen species; RNS, reactive nitrogen species; TdT, terminal deoxynucleotidyl transferase; HRP, horse radish peroxidase; Hsp, heat shock proteins.

http://dx.doi.org/10.1016/j.abb.2014.06.008
0003-9861/

contain 3 different HSFs known as HSF1, HSF2 and HSF4. HSF1 is a major HSF, which mediates the regulation of several heat shock genes while HSF2 is involved in the differentiation and developmental processes. HSF4 is involved in the postnatal expression of Hsp [16]. The phosphorylation status of sHsp is important for determining their chaperone activity and cytoprotective functions [17,18]. Hsp27 is phosphorylated at serine positions 82 (S82), 72 (S72) and 15 (S15) while αBC at 59 (S59), 45 (S45) and 19 (S19). The p38 mitogen activated protein kinase (MAPK)/MAPK activated protein-2 is responsible for phosphorylation at S82, S72, S15 of Hsp27 and S59 of αBC [19,20]. While S45 of αBC is phosphorylated by p44/42 MAP kinase (ERK), kinase responsible for phosphorylation of S19 of αBC is unknown.

Previously we have reported the elevated expression of α-crystallins, two prominent members of sHsp, in various tissues including heart in diabetic rats [21]. Recently we have also observed induction of some members of sHsp family and their phosphoregulation in retina [22] and lens [23] of diabetic rat model. However, the effect of chronic hyperglycemia on expression of sHsp family members, kinase mediated phosphoregulation and their involvement in cytoskeletal protection and apoptosis in experimental diabetic heart has not been examined. In the present study, for the first time, we investigated the response of sHsp family members in chronic hyperglycemia and their translocation from cytosol to striated sarcomeres in cardiac myofibers and role in apoptotic cell death in diabetic rat heart.

Materials and methods

Materials

Streptozotocin (STZ), Tri-reagent, TritonX-100 (TritonX), acrylamide, bis-acrylamide, ammonium persulphate, β-mercaptoethanol, SDS, TEMED, PMSF, aprotinin, leupeptin, pepstatin, anti-actin antibody (Cat.No-A5060), horse radish peroxidase (HRP) conjugated anti-rabbit (A6154) and anti-mouse (A9044) secondary antibodies were purchased from Sigma Chemicals (St. Louis, MO, USA). Nitrocellulose membrane was obtained from Pall Corporation (Pensacola, FL, USA). Anti-Hsp27 (MA3-014), anti-Hsp20 (PA1-29447), anti-HSF1 (PA3-017), and specific antibodies recognizing three phosphorylated residues S59 (PA1-012), S45 (PA1-011), and S19 (PA1-010) of αBC were obtained from Thermo Scientific-Pierce (Rockford, IL, USA). Anti-αBC antibody was produced in the rabbit as reported previously [24]. Anti-MKBP (18-821-485217), anti-Hsp22 (18-821-485201) were obtained from Genway (San Diego, CA, USA). Anti-p38MAPK (#9212S), anti-p-p38MAPK (#9211S), anti-p44/42 MAPK (#4695), anti-p-p44/42 MAPK (#4370), anti-cleaved caspase-3 (#9661S), anti-pS82-Hsp27 (#2401S), anti-Hsp70 (#4872) antibodies were purchased from Cell Signaling Technology, Inc. (Beverly, MA). Anti-HspB3 (sc-104935), anti-HSF2 (sc-13056), anti-HSF4 (sc-19864), anti-Bax (sc-6236), anti-Bcl-2 (sc-492), anti-desmin (sc-23879) and HRP conjugated anti-goat (sc-2020) secondary antibodies were purchased from Santa Cruz Biotechnology, Inc. (Dallas, Texas, USA). All primers were procured from Integrated DNA Technologies (Coralville, IA, USA). Alexafluor-488 conjugated anti-rabbit (A-11008) and Alexafluor-555 conjugated anti-mouse (A-21427) antibodies were obtained from Molecular Probes, Inc. (Eugene, OR, USA).

Animal care and experimental conditions

Three-month-old male WNIN (Wistar-NIN) rats with average body weight of 230 ± 14 g were obtained from National Center for Laboratory Animal Sciences, National Institute of Nutrition, Hyderabad, India, and maintained at a temperature of 22 ± 2 °C, 50% humidity, and 12-h light/dark cycle as described previously and the heart tissues of the same animals were used [22]. The control rats (n = 10) received 0.1 M sodium citrate buffer, pH 4.5, as a vehicle, whereas the experimental rats received a single intraperitoneal injection of STZ (35 mg/kg bw) in the same buffer. At 72 h after STZ injection, fasting blood glucose levels were monitored and animals with blood glucose levels >150 mg/dL were considered for the experiment (n = 10). Control and diabetic animals were fed with AIN-93 diet *ad libitum*. Body weight and blood glucose concentration of each animal were measured weekly. At the end of 12 weeks, rats were fasted overnight and sacrificed by CO2 asphyxiation. Institutional and national guidelines for the care and use of animals were followed, and all experimental procedures involving animals were approved by the Institutional Animal Ethical Committee (IAEC) of the National Institute of Nutrition.

Biochemical estimations

Glucose and glycosylated hemoglobin (HbA1c) in blood were measured by the glucose oxidase -peroxidase (GOD–POD) method and ion-exchange resin, respectively, using commercially available kits (Biosystems, Barcelona, Spain).

Quantitative real-time PCR (qRT-PCR)

Total RNA was extracted from control and diabetic rat heart using Tri-reagent according to the manufacturer instructions. Isolated RNA was further purified by RNeasy Mini Kit (Qiagen, USA) and quantified by measuring the absorbance at 260 and 280 nm on ND1000 Spectrophotometer (NanoDrop Technologies, Delaware, USA). Two to 4 μg of total RNA was reverse transcribed using High Capacity cDNA Reverse Transcription kit (Applied Biosystems, Warrington, UK). Reverse transcription reaction was carried out using thermocylcer (ABI-9700) and the reaction conditions were as follows: initial temperature for 10 min at 25 °C, followed by 37 °C for 120 min and inactivation of reverse transcriptase at 84 °C for 5 min. Real-time PCR (ABI-7500) was performed in triplicates with 25 ng cDNA templates using SYBR green master mix (Applied Biosystems, Warrington, UK) with gene specific primers (Table. 1). Normalization and validation of data were carried using β-actin as an internal control and data were compared between control and diabetic samples according to comparative threshold cycle ($2^{-\Delta\Delta ct}$) method as reported previously [22,25].

Whole tissue lysate preparation

Heart tissue (100–200 mg) was homogenized in TNE buffer (pH 7.5) containing 20 mM Tris, 100 mM NaCl, 1 mM EDTA, 1 mM DTT and protease inhibitors. Homogenization was performed on ice using a glass homogenizer and the homogenate was centrifuged at 14,000g at 4 °C for 20 min. The protein concentrations were measured by Bradford reagent (Bio-Rad, Hercules, CA).

SDS–PAGE and immunoblotting

Equal amounts of protein were subjected to 12% SDS–PAGE and transferred to nitrocellulose membranes (0.22 μm pore size) by western blot transfer system (Bio-Rad, USA) at a voltage of 40 V for 2 h. Nonspecific binding was blocked with 5% nonfat dry milk powder in PBST (20 mM phosphate buffer; pH 7.2, 137 mM NaCl, 0.1% Tween 20) and incubated overnight at 4 °C with monoclonal anti-Hsp27 (mouse, 1:500), anti-p44/42 MAPK (rabbit, 1:1000), anti-p-p44/42 MAPK (rabbit, 1:2000), polyclonal anti-pS82-Hsp27 (1:1000), anti-αBC (rabbit, 1:3000), anti-pS59, pS45, pS19-αBC (rabbit, 1:2000), anti-HspB3 (goat; 1:1000), anti-Hsp20