Pharmacological Research 116 (2017) 45–56

Contents lists available at ScienceDirect

Pharmacological Research

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

Invited Perspective

Insights into the role of maladaptive hexosamine biosynthesis and *O*-GlcNAcylation in development of diabetic cardiac complications

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ARTICLE INFO

Article history:
Received 8 June 2016
Received in revised form 28 October 2016
Accepted 13 December 2016
Available online 14 December 2016

Keywords:
O-GlcNAcylation
Diabetic cardiomyopathy
Hyperglycemia
Diastolic function
Cardiac remodeling

ABSTRACT

Diabetes mellitus significantly increases the risk of heart failure, independent of coronary artery disease. The mechanisms implicated in the development of diabetic heart disease, commonly termed diabetic cardiomyopathy, are complex, but much of the impact of diabetes on the heart can be attributed to impaired glucose handling. It has been shown that the maladaptive nutrient-sensing hexosamine biosynthesis pathway (HBP) contributes to diabetic complications in many non-cardiac tissues. Glucose metabolism by the HBP leads to enzymatically-regulated, O-linked attachment of a sugar moiety molecule, β-*N*-acetylglucosamine (*O*-GlcNAc), to proteins, affecting their biological activity (similar to phosphorylation). In normal physiology, transient activation of HBP/*O*-GlcNAc mechanisms is an adaptive, protective means to enhance cell survival; interventions that acutely suppress this pathway decrease tolerance to stress. Conversely, chronic dysregulation of HBP/*O*-GlcNAc mechanisms has been shown to be detrimental in certain pathological settings, including diabetes and cancer. Most of our understanding of the impact of sustained maladaptive HBP and *O*-GlcNAc protein modifications has been derived from adipose tissue, skeletal muscle and other non-cardiac tissues, as a contributing mechanism to insulin resistance and progression of diabetic complications. However, the long-term consequences of persistent activation of cardiac HBP and *O*-GlcNAc are not well-understood; therefore, the goal of this timely review is to highlight current understanding of the role of the HBP pathway in development of diabetic cardiomyopathy.

Abbreviations: AGE, Advanced glycation end product; Azaserine, *O*-diazoacetyl-L-serine; BAG, Benzyl-2-acetamido-2-deoxy-a-D-galactopyranoside; cAMP, Cyclic adenosine-3′5′-monophosphate; CaMKII, Ca^{2+}/calmodulin-dependent protein kinase II; CRAC, Ca^{2+} release activated Ca^{2+} channel; CTD, C terminal domain; DAG, Diacylglycerol; DON, 6-diazo-5-oxonorleucine; EC, Excitation contraction; GLUT-4, Glucose transporter-4; eNOS, Endothelial nitric oxide synthase; HBP, Hexosamine biosynthesis pathway; GFAT, Glutamine fructose-6-phosphate amidotransferase; HF, Heart failure; IP_3, Inositol triphosphate; IP_3-1R, Inositol triphosphate-1 receptor; ISO, Isoprenaline; LV, Left ventricle/ventricular; MHC, Myosin heavy chain; MLC, Myosin light chain; mOGT, Mitochondrial OGT; NButGT, 1,2-dideoxy-2′-propyl-α-D-glucopyranoso-[2,1-D]-Δ2′-thiazoline; ncOGT, Nucleocytoplasmic OGT; NCX, Na^+/Ca^{2+} exchanger; NOS, Nitric oxide synthases; NFAT, Calcineurin-nuclear factor of activated T cell; OGA, *O*-GlcNAcase; *O*-GlcNAc, *O*-linked β-N-acetylglucosamine; OGT, *O*-GlcNAc transferase; PE, Phenylephrine; PIP_2, Phosphatidylinositol (4,5)-biphosphate; PIP_3, Phosphatidylinositol (3,4,5)-triphosphate; PKC, Protein kinase C; PLB, Phospholamban; PLC, Phospholipase C; PUGNAc, O-(2-acetamido-2-deoxy-D-glucopyranosylidene)amino *N*-phenyl carbamate; ROS, Reactive oxygen species; SERCA, Sarcoplasmic reticulum Ca^{2+}-ATPase; 5S-GlcNAc, 5-thioglucosamine; SOD, Superoxide dismutase; Sp1, Specificity protein 1; STIM1, Stromal interaction molecule 1; STZ, Streptozotocin; T1DM, Type 1 diabetes mellitus; T2DM, Type 2 diabetes mellitus; Thiamet G, O-(2-acetamido-2-deoxy-D-glucopyranoseylidene); TIMP, Tissue inhibitor of metalloproteinases; TT04, 2H-1, 3-thiazine-6-carboxylic acid, 2-[(4-chlorophenyl) imino] tetrahydro-4-oxo-3-(2-tricyclo[3.3.1.13, 7] dec-1-ylethyl-); UDP-GlcNAc, Uridine diphosphate-*N*-acetylglucosamine.

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http://dx.doi.org/10.1016/j.phrs.2016.12.016
1043-6618/

1. Introduction

1.1. Overview of diabetic cardiomyopathy

Diabetes Mellitus is a chronic disease projected to affect 600 million adults worldwide by 2035 [1,2]. Diabetes is caused by deficiency of, or resistance to, the hormone insulin, resulting in a hyperglycemic state. The two predominant forms of diabetes include type 1 diabetes mellitus (T1DM, previously known as juvenile or insulin-dependent diabetes mellitus) and type 2 diabetes mellitus (T2DM, also referred to as non-insulin-dependent diabetes mellitus). Of the many diabetic complications that arise as a consequence of poor glucose control and hyperglycemia, cardiovascular complications account for significant morbidity and mortality [2,3]. Diabetes is a distinct primary disease phenotype of impaired myocardial function that can develop independent of concomitant comorbidities, such as coronary artery disease and atherosclerosis. It is characterized by impairment of left ventricular (LV) diastolic function, cardiomyocyte hypertrophy, LV fibrosis and elevated LV reactive oxygen species (ROS) levels [2,3]. The etiology of diabetic cardiomyopathy is distinct from other causes of LV dysfunction, as it is characterized initially by LV diastolic (rather than systolic) dysfunction, where relaxation of the cardiac muscle following contraction is prolonged [2,4]. Diastolic dysfunction is estimated to be present in at least 50% of asymptomatic, normotensive patients with well-controlled diabetes, preserved LV ejection fraction and no overt coronary artery disease [5]. Diastolic dysfunction raises the pressure gradient of blood in the pulmonary vessels, which causes fluid to leak from these vessels into the lung alveoli, causing pulmonary edema. This condition impairs oxygenation of blood in the lungs, causing shortness of breath, and even death, if the condition is not diagnosed properly.

Much of the impact of diabetes on the heart can be attributed to hyperglycemia and increased ROS, as reviewed previously [2,6]. Mechanisms that contribute to hyperglycemia includes increased flux of glucose and other sugars through the polyol pathway [7], increased intracellular formation of advanced glycation end products [8], activation of protein kinase C (PKC) [9], and increased flux through the hexosamine biosynthesis pathway (HBP) [6,10]. With emerging evidence that increased maladaptive glucose metabolism through the HBP plays an important role in many diabetes complications [11], this review provides a timely summary of our present understanding of its role in the development of diabetic cardiomyopathy.

2. Hexosamine biosynthesis pathway

2.1. Overview of HBP/O-GlcNAcylation

Upon entering a cell, glucose is phosphorylated to glucose-6-phosphate, and metabolized to fructose-6-phosphate, permitting entry into the accessory pathways of glucose metabolism, glycolysis and gluconeogenesis. One such pathway is the O-linked β-*N*-acetylglucosamine (*O*-GlcNAc) modification of proteins (or HBP pathway), as illustrated in Fig. 1. The post-translational, dynamic addition and removal of *O*-GlcNAc to target proteins, including regulators of transcription and translation, cytoskeletal and other nucleocytoplasmic proteins, has emerged as a potential key player in progression of pathophysiology [12–15]. In adipocytes, it has been that under normal physiology, the HBP consumes 2–5% of cellular glucose [16]; however, a direct measure of glucose flux through this pathway has yet to be reported in either cardiomyocytes or the intact heart. To date, our knowledge of *O*-GlcNAc protein modification has largely been derived from studies of adipose tissue, skeletal muscle, neuronal and other non-cardiac tissues [12,15,17].

The HBP comprises four sequential enzymatic reactions that convert fructose-6-phosphate to uridine diphosphate-N-acetylglucosamine (UDP-GlcNAc), the monosaccharide donor for *O*-GlcNAc protein modifications (Fig. 1). The first reaction is the rate-limiting conversion of fructose-6-phosphate to glucosamine-6-phosphate by L-glutamine-fructose-6-phosphate amidotransferase (GFAT), with concomitant conversion of glucosamine to glutamine [16,18]. The second reaction is the conversion of glucosamine-6-phosphate to *N*-acetylglucosamine-6-phosphate by glucosamine-6-phosphate acetyl-transferase, using acetyl-CoA as a substrate [19]. The penultimate reaction converts *N*-acetylglucosamine-6-phosphate to *N*-acetyleglucosamine-1-phosphate, using phosphoglucomutase. Finally, pyrophosphorylase catalyzes the conjugation of *N*-acetylglucosamine to a uridine nucleotide to generate UDP-GlcNAc, which serves as the monosaccharide donor for *O*-GlcNAcyation [15,20,21]. During this process, *O*-GlcNAc transferase (OGT) attaches *O*-GlcNAc to protein serine & threonine residues ('*O*-GlcNAcylation') of the affected protein. In contrast, β-N-acetylglucosaminidase (or '*O*-GlcNAc-ase', OGA) removes *O*-GlcNAc (Fig. 1) [12,15,20–22]. Interestingly, nutrient-derived glucose, glutamine, acetyl-CoA, and glucosamine all feed into the HBP at different points linking the pathway to amino acid metabolism, lipolysis, lipogenesis, and glucose oxidation [14,23–28].

2.2. Identification of O-GlcNAc-modified proteins

Techniques to quantify *O*-GlcNAc modification of proteins continue to develop [29,30]. Recently, Vercoutter-Edouart and colleagues have reviewed the list of proteins modified by *O*-GlcNAc (~1000 were identified) by a proteomics approach [19]. The transient nature of its addition and removal from proteins, its lack of charge, small size, and its similarity to other small sugars, all pose a number of challenges to identify and characterize *O*-GlcNAc related targets [31]. This protein modification was first detected on lymphocytes with radio-labelled galactose tagging [32]. Antibody binding and fluorophore labeling remain preferred techniques for identifying *O*-GlcNAc modified proteins. The CTD (C-terminal domain) 110.6 clone is the most commonly-used commercial IgM pan-GlcNAc antibody [33], although in some limited settings m this can cross-react with *N*-GlcNAc-modified glycoproteins under glucose deprivation [26,34]. Furthermore, the IgM antibody cannot easily be used for immunoprecipitation because it will not bind to protein A/G-agarose beads [34]. The first generation of monoclonal IgG antibodies for studying *O*-GlcNAc (e.g. RL2, from Millipore) only however recognize a smaller subset of *O*-GlcNAcylated proteins, less than CTD110.6. Three recently developed monoclonal antibodies [clone 18B10.C7 [3], clone $9D_1{\cdot}E_4$ [10], and clone $1F_5{\cdot}D_6$ [14]] have recently become commercially available [15], but these still require further validation in experimental settings. These antibodies exhibit relatively low binding affinities, and thus may only be employed for the detection of multiple modified, higher molecular weight or highly abundant proteins.

An additional consideration is that the *O*-GlcNAc modification is not sufficiently stable for conventional tandem mass spectrometry or Western blot; the GlcNAc moiety is too easily lost from the serine/threonine side chain upon collision with gas molecules, precluding direct detection of the modification site. More expensive, highly-specialized mass spectrometry instrumentation is required for identifying site-specific *O*-GlcNAc protein modifications [35–39]. Developments in chemoenzymatic techniques, with a chemically-tagged *O*-GlcNAc moiety, have enabled mass-spectrometry-based proteomic analysis of *O*-GlcNAc-modified proteins. In addition, metabolic-labeling techniques of chemically-