Glucotoxic conditions induce endoplasmic reticulum stress to cause caspase 3 mediated lamin B degradation in pancreatic β-cells: Protection by nifedipine

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ABSTRACT

Nuclear lamins form the lamina on the interior of the nuclear envelope, and are involved in the regulation of various cellular processes, including DNA replication and chromatin organization. Despite this evidence, little is known about potential alterations in nuclear metabolism, specifically lamin structure and integrity in isolated β-cells subjected to stress conditions, including chronic exposure to hyperglycemia (i.e., glucotoxicity). Herein, we investigated effects of glucotoxic conditions on the catalytic activation of caspase 3 and the associated degradation of one of its substrate proteins, namely lamin-B. We report that incubation of insulin-secreting INS-1 832/13 cells, normal rat islets or human islets under glucotoxic conditions (20 mM; 12–48 h) results in the degradation of native lamin B leading to accumulation of the degraded products in non-relevant cellular compartments, including cytosol. Moreover, the effects of high glucose on caspase 3 activation and lamin B degradation were mimicked by thapsigargin, a known inducer of endoplasmic reticulum stress (ER stress). Nifedipine, a known blocker of calcium channel activation, inhibited high glucose-induced caspase 3 activation and lamin B degradation in these cells. 4-Phenyl butyric acid, a known inhibitor of ER stress, markedly attenuated glucose-induced CHOP expression (ER stress marker), caspase 3 activation and lamin B degradation. We conclude that glucotoxic conditions promote caspase 3 activation and lamin B degradation, which may, in part, be due to increased ER stress under these conditions. We also provide further evidence to support beneficial effects of calcium channel blockers against metabolic dysfunction of the islet β-cell induced by hyperglycemic conditions.

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1. Introduction

Apoptosis is the process of programmed cell death that is required for the maintenance of tissue homeostasis. It is characterized by specific changes in the morphology of the cell. The cell undergoes shrinkage, blebbing (bulges in the plasma membrane), nuclear fragmentation and chromatin condensation [1]. Basically, it is a natural process that balances cell growth and removal of damaged or injured cells. It is well established that β-cell failure is a characteristic feature of Type 1 and 2 diabetes. In both types of the disease, apoptosis is the most likely form of cell death. Therefore, it is necessary to study the intracellular events that the pancreatic β-cell undergoes during apoptosis, and understanding of such events will aid in searching for a possible drug target for the management and/or prevention of the disease [2,3].

The caspase (cysteine dependent aspartate-directed proteases) family is one of the most important factors in the apoptotic pathway. Many proteins in the cell including a large number of structural proteins are cleaved by activated caspases which culminates in apoptosis of the cell. The caspase 8, 9 and 10 are initiator caspases whereas caspase 3, 6 and 7 are effector caspases. The initiator caspases cleave and activate the effector caspases leading to cell death [1]. These caspases exist in the form of inactive zymogens (also referred to as pro-caspases), which are activated by the process of cleavage by other upstream proteases or by auto- or trans-activation [4]. In the recent years a variety of substrates of caspases have been recognized. Caspase-dependent degradation of nuclear lamins has been identified as a precursor to nuclear collapse in programmed cell death [5]. The nuclear lamina lines the

interior of the nuclear envelope and is composed of three proteins; lamins A, B and C. The A type lamins, which include lamins A and C, are products of alternative splicing of the same gene LMNA whereas lamin B is encoded by the LMNB1 gene [6]. These lamins are type V intermediate filament proteins and are lined up on the inner face of the inner nuclear membrane. The nuclear lamina is a fundamental part of major nuclear activities, namely mitosis, chromatin organization and in DNA replication. Besides, lamins also play key functional roles in providing structural support thereby contributing to the nuclear architecture [7].

Degradation of lamins leads to the breakdown of nuclear lamina which is a preliminary stage of apoptosis [8] as this is followed by DNA degradation and chromatin condensation [9]. Previous results from our laboratory indicated that IL-1β treatment causes an increase in lamin B degradation mediated by caspases [10]. In addition, a recent study highlighted the involvement of caspase 3 in the breakdown of the nuclear matrix by cleaving nuclear lamin B. It has been stated that this cleavage probably occurs by activation of caspase 3 directly or by other downstream proteases [11]. Recent evidence in PC12 and rat cortical cells also implicates cell apoptosis via caspase 3 activation and cleavage of lamin-B under lipotoxic conditions induced by saturated fatty acids, such as palmitic acid [12].

It is well established that chronic exposure of isolated β-cells to hyperglycemic conditions leads to metabolic dysfunction and demise [13,14]. Several intracellular signaling events have been identified as causal to HG-induced metabolic dysregulation of the islet. These include endoplasmic reticulum (ER) and oxidative stress. It has been suggested that both oxidative and ER stress lead to mitochondrial dysfunction, cytochrome-C release, and caspase activation resulting in the demise of the β-cell [15]. Despite this growing body of evidence, very little is known in the context of the islet β-cell, on potential detrimental effects of glucotoxic conditions on caspase activation and associated degradation of their respective substrate proteins. The overall objective of this study therefore is to investigate the role of caspase 3 on the degradation of nuclear lamins, specifically lamin-B in insulin-secreting INS-1 832/13 cells, rat islets and human islets under glucotoxic conditions. Our findings provide the first evidence to implicate significant caspase 3 mediated degradation of lamin B in these cells under glucotoxic environment. We also provide evidence to suggest novel roles for ER stress in glucose-induced caspase 3 activation and lamin B degradation, and associated alterations in the subcellular distribution of lamin B under glucotoxic conditions.

2. Materials and methods

2.1. Materials

Antisera directed against lamin-B, procaspase-3, and cleaved caspase-3 (Asp 175), CHOP were obtained from Cell Signaling (Danvers MA and Santa Cruz Biotechnology, Santa Cruz, CA). Anti-β Actin was from Sigma–Aldrich (St. Louis, MO). Anti-mouse IgG and anti-rabbit IgG conjugated to horseradish peroxidase were from GE Healthcare UK. Glucose, nifedipine, thapsigargin, 4-phenylbutyric acid were obtained from Sigma–Aldrich (St. Louis, MO). Enhanced chemiluminescence (ECL) kit was from Amersham Biosciences (Piscataway, NJ). All other reagents were from Sigma–Aldrich (St. Louis, MO) unless stated otherwise.

2.2. Insulin-secreting INS-1 832/13 cells, rat islets, human islets and culture conditions

INS-1 832/13 cells were cultured in RPMI-1640 medium containing 10% heat inactivated FBS supplemented with 100 IU/ml penicillin and 100 IU/ml streptomycin, 1 mM sodium pyruvate,

50 μM 2-mercaptoethanol and 10 mM HEPES (pH 7.4). The cultured cells were subcloned twice weekly following trypsinization and passages 53–61 were used for the study. Islets from normal 6-week-old male Sprague–Dawley rats (Harlan Laboratories, Oxford, MI) were isolated by the collagenase digestion method [16]. Human islets and islet culture medium were obtained from Prodo Laboratories, Irvine, CA. All protocols, including isolation of pancreatic islets from rats, were reviewed and approved by the Wayne State University and John D. Dingell VA Medical Center Institutional Animal Care and Use Committee.

INS-1 832/13 cells and normal rat islets were incubated in the presence of low (2.5 mM) and high glucose (20 mM or 30 mM in case of human islets) for 12–48 h as indicated in the text. Low and high glucose solutions were prepared by supplementing glucose to glucose-free medium where appropriate. At the end of the incubation period the cells were harvested and lysed in RIPA buffer containing 1 mg/ml protease inhibitor cocktail, 1 mM NaF, 1 mM PMSF and 1 mM Na$_3$VO$_4$.

2.3. Isolation of subcellular fractions

Extraction of cytosolic, membrane/organelle and nucleic protein fraction was carried out as per the manufacturer's instructions using the ProteoExtract® Subcellular Proteome Extraction Kit. Briefly, INS-1 832/13 cells were incubated with low (2.5 mM) and high (20 mM) glucose for 24 h. The cells were scraped and suspended in wash buffer and pelleted by centrifugation for 10 min at 300 × g at 4 °C. The pellet obtained was then resuspended in the extraction buffer-I and protease inhibitor cocktail, provided in the kit. After incubation for 10 min at 4 °C the cells were centrifuged for 10 min at 1000 × g, the supernatant obtained was the cytosolic fraction. The pellet was then resuspended in Extraction buffer-II and protease inhibitor cocktail and incubated for 30 min and then centrifuged for 10 min at 6000 × g. The supernatant thus obtained was the membrane/organelle fraction. Finally the pellet was resuspended in extraction buffer-III, protease inhibitor cocktail and benzonase, incubated for 10 min at 4 °C and centrifuged at 6800 × g for 10 min. The supernatant obtained was the nucleic protein fraction and the remaining pellet, resuspended in buffer IV and protease inhibitor cocktail was the cytoskeletal matrix protein fraction.

2.4. Western blotting

Cellular lysate proteins (30–50 μg/lane) were separated by SDS-PAGE on 10% (w/v) polyacrylamide mini gels and electro transferred to a nitrocellulose membrane. The membranes were blocked with 5% milk in 10 mM Tris–HCl, pH 7.6, 1.5 M NaCl and 0.1% Tween 20 followed by incubation with primary antibodies (lamin B—1:250, cleaved caspase 3—1:400) in TBS-T containing 5% BSA at room temperature for 1 h and washed 5 times for 5 min each with TBS-T. The membrane was then incubated with corresponding secondary antibodies conjugated to horseradish peroxidase (1:1000) in 5% non-fat dry milk in TBS-T at room temperature for 1 h. After washing, the protein signal was enhanced by chemiluminescence system and developed using Kodak Pro Image 400 R (New Haven, CT) and Carestream Molecular Imaging Software was used to measure the band density. The same blots were used to probe for β-actin to ensure equal loading and transfer of proteins.

2.5. Statistical analysis

The statistical significance of the differences between the experimental conditions was determined by *t*-test or ANOVA where appropriate. *p* value < 0.05 was considered significant.