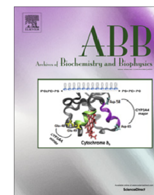
Archives of Biochemistry and Biophysics 542 (2014) 7–13

Contents lists available at ScienceDirect

Archives of Biochemistry and Biophysics

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



Hyperglycaemia modifies energy metabolism and reactive oxygen species formation in endothelial cells *in vitro*

Dorota Dymkowska *, Beata Drabarek, Paulina Podszywałow-Bartnicka, Joanna Szczepanowska, Krzysztof Zabłocki

Nencki Institute of Experimental Biology PAS, Warsaw, Poland

ARTICLE INFO

Article history:
Received 29 May 2013
and in revised form 6 November 2013
Available online 1 December 2013

Keywords:
Endothelial cells
Mitochondria
Reactive oxygen species
NADPH oxidase

ABSTRACT

There is significant evidence for an involvement of reactive oxygen species (ROS) in the pathogenesis of diabetic vascular complications through many metabolic and structural derangements. However, despite the advanced knowledge on the crucial role of ROS in cardiovascular damage, their intracellular source in endothelial cells exposed to high concentrations of glucose has not been precisely defined. Moreover, the molecular mechanism of action of elevated glucose on mitochondria has not been fully elucidated. The main aim of this study was to describe changes in the mitochondrial metabolism of human umbilical vein endothelial cells (HUVECs) treated with high glucose concentrations and to indicate the actual source of ROS in these cells.

HUVECs exposed to 30 mM glucose exhibited an increased content of vascular adhesive molecule-1 (VCAM-1) and an excessive ROS production. Faster oxygen consumption and increased abundance of selected respiratory complexes coexist with slightly declined mitochondrial membrane potential and substantially elevated amount of uncoupling protein-2 (UCP2). Inhibition of NADPH oxidase (NOX) and modification of mitochondrial ROS generation with a mitochondrial uncoupler or respiratory chain inhibitors allowed concluding that the major source of ROS in HUVECs exposed to hyperglycaemic conditions is NOX. The mitochondrial respiratory chain seems not to participate in this phenomenon.

© 2013 Elsevier Inc. All rights reserved.

Introduction

Reactive oxygen species (ROS)[1] generated during hyperglycaemia are postulated to be one of the most important contributors to the development and progression of diabetic vascular complications and often associated with endothelial dysfunction. Previous studies have indicated that not only prolonged exposure to hyperglycaemic conditions but also transient acute hyperglycaemia impairs endothelial function, as evidenced by an impaired ability to nitric oxide release [1]. Hyperglycaemia also alters the intracellular redox state which regulates the activity of several transcription factors, including nuclear factor κB (NF-κB), activator protein-1 (AP-1) and c-jun, implicated in the inducible expression of a wide variety of genes involved in oxidative stress and cellular stress response mechanisms [2]. For example, Bakkar et al. [3] showed that the activation of NF-κB stimulated mitochondrial biogenesis and function, suggesting participation of so-called alternative signalling pathway in these processes. Activation of this pathway, mediated by NF-κB-inducing kinase, results in displacement of p52 protein to the nucleus and contributes to the mitochondrial mass increase. Moreover, an elevated serum glucose concentration decreases endothelial nitric oxide synthase (eNOS) mRNA and protein level in human endothelial cells by modulating mitochondrial ROS production. This, in turn, reduces nitric oxide (NO) availability [4].

Although several sources of reactive oxygen species may be involved in the response of endothelial cells to hyperglycaemia, NADPH oxidases (NOX) are postulated to be the most significant producers of ROS in the cardiovascular system. However, the mitochondrial electron transport needs also to be considered as an important source of reactive oxygen species.

It should be noted that ROS, produced in a tightly controlled mode by NOX and the mitochondrial respiratory chain, could act as a physiological regulator of intracellular signalling pathways leading to cytokine secretion, vascular proliferation, hypertrophy and remodelling. On the other hand, an excessive ROS generation may be involved in atherosclerosis progression [5]. It also results in oxidative cell damage due to membrane lipid peroxidation, DNA strand breaks, and protein oxidation, all of which are

* Corresponding author. Address: Nencki Institute of Experimental Biology, 3 Pasteura Str. 02-093 Warsaw, Poland. Fax: +48 22 8225342.

E-mail address: d.dymkowska@nencki.gov.pl (D. Dymkowska).

[1] *Abbreviations used:* ROS, reactive oxygen species; HUVEC, human umbilical vein endothelial cells; VCAM-1, vascular adhesive molecule-1; ICAM-1, intracellular adhesive molecule-1; UCP2, uncoupling protein-2; eNOS, endothelial nitric oxide synthase; NOXNADPH oxidase, DPI diphenyleneiodonium - NADPH oxidase inhibitor.

0003-9861/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.abb.2013.11.008

important processes contributing to endothelial dysfunction and cell death [6].

There is convincing evidence that hyperglycaemia leads to an activation of cellular stress pathways, and mitochondrial participation in this process is of particular interest. The high degree of endothelial mitochondria damage seen in atherosclerosis is one observation supporting such a correlation [7]. Mitochondrial ROS/RNS (reactive oxygen/nitrogen species)-evoked dysfunction results in a loss of control of metabolic function, activation of stress pathways and progression to vascular damage. However, still little is known about the changes in the metabolism of mitochondria in endothelial cells challenged by a high concentration of glucose. Some data indicate an increase in UCP2 level, which may protect endothelial cells from oxidative damage by slightly reducing the mitochondrial membrane potential and, consequently decreasing ROS formation. In fact, in primary aortic endothelial cells exposed to glucose at an extremely high concentration, UCP2 overexpression reduced intracellular ROS production as well as enhanced eNOS phosphorylation [8]. On the other hand, diabetes-associated metabolic disorders in the cardiovascular system may involve both mitochondrial dysfunction and the up-regulation of NADPH oxidase which may be the major source of ROS [9].

Thus, ROS may originate in endothelial cells mainly from the activity of NOX and the mitochondrial respiratory chain. For example, mitochondrial superoxide formation in bovine aortic endothelial cells (BAEC) was confirmed by chemical depletion of mitochondrial DNA (rho^0 cells) or after treatment with mitochondria-targeted antioxidants [10]. Alternatively, increased ROS formation in human aortic endothelial cells was attributed to endothelial nitric oxide synthase activity [11]. In immortalized EA.hy926 cells hyperglycaemia-induced ROS appear to be produced by both NADPH oxidase and mitochondrial respiratory chain [12].

In this paper the mechanism of glucose-induced ROS formation in HUVEC cells is investigated. Selective inhibition of NOX and uncoupling of oxidative phosphorylation/inhibition of the respiratory chain allowed us to conclude that the increased ROS generation under hyperglycaemic conditions is dependent on NOX rather than on the mitochondrial electron transport. Moreover, the influence of hyperglycaemia on the mitochondrial energy metabolism and the proteins involved in oxidative phosphorylation and oxidative stress regulation was also investigated. Understanding of the mechanism(s) linking hyperglycaemia and energy metabolism of endothelial cells as well as a precise identification of the intracellular ROS source under such pathological conditions could help to design a strategy for cell protection against the deleterious effect of increased serum glucose concentration.

Methods

Cell culture

Human umbilical vein endothelial cells purchased from Lonza (Walkersville, MD USA) were grown in Endothelial Cell Growth Medium BulletKit®-2 (EGM-2 BulletKit, Lonza) at 37 °C in the atmosphere of 5% CO_2 and 95% air. The culture medium was supplemented with conveniently packaged as single-use aliquots called SingleQuots, containing as follows: human recombinant epidermal growth factor, human fibroblast growth factor, vascular endothelial growth factor, ascorbic acid, hydrocortisone, human recombinant insulin-like growth factor, heparin, 2% foetal bovine serum and gentamicin with amphotericin (Lonza). Cells were passaged every 2 days. Passages 2 through 5 were used for all experiments. Confluent cells were grown in normal (5 mM) or high (30 mM) glucose conditions for 48 h.

Oxygen consumption

Cellular respiration rate was measured polarographically as oxygen uptake using OROBOROS Oxygraph-2k (OROBOROS® INSTRUMENTS GmbH, Austria) at 37 °C. Confluent cells grown in 10 cm tissue culture dishes were trypsinized, centrifuged and resuspended in 2 ml PBS pre-warmed to 37 °C (approximate density 0.5 mg protein/ml). Prior to the assay calibration procedure and background correction was performed according to the manufacturer's protocol. Oxygen consumption was measured in the presence of sequentially added respiratory substrates (1 mM pyruvate, 5 mM glucose, Sigma), oligomycin (0.1 μg/ml, Sigma) and CCCP (0.5 μM, Sigma). Respiration rate was normalized to the amount of protein in the assay.

Mitochondrial membrane potential

Mitochondrial membrane potential was measured fluorimetrically with 5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethylbenzimidazolcarbocyanine iodide (JC-1, Molecular Probes, Invitrogen) according to the method of Cossarizza et al. [13]. Confluent cells growing in 24-well culture plates were stained with JC-1 (5 μM final concentration in the culture medium) and incubated for 15 min at 37 °C in the dark. Then, the cells were rinsed three times with the culture medium followed by PBS. Finally, 0.5 ml PBS was added to each well. The cells with completely deenergized mitochondria due to addition of 2 μg/ml of valinomycin plus 5 μM CCCP were used as a control. Fluorescence was measured with a laser scanning cytometer iCYS equipped with argon laser (excitation 488 nm). The data were presented as the ratio of orange (energized) to green (deenergized) fluorescence.

Reactive oxygen species

ROS were measured with carboxy-2′,7′-difluorodihydrofluorescein diacetate ($DFFH_2$-DA, Molecular Probes, Invitrogen). Confluent cells grown in 12-well plates were stained with 10 μM $DFFH_2$-DA in PBS for 30 min at 37 °C in the dark. Then, fluorescence was measured at 485 nm excitation and 520 nm emission wavelengths using Infinite 200 micro plate reader (Tecan). Total green fluorescence was normalized to the amount of protein in each well.

Mitochondrial mass

Mitochondrial mass was estimated fluorimetrically with Mitotracker Green (Molecular Probes, Invitrogen). Confluent cells growing in 24-well plates were stained with this dye at 100 nM concentration in PBS for 20 min at 37 °C. Then, the cells were rinsed three times with the culture medium and then with PBS. Finally, 0.5 ml PBS was added to each well. Fluorescence was measured with laser scanning cytometer iCYS equipped with argon laser (excitation 488 nm). Total green fluorescence was normalized to the amount of protein in each well.

Cell lysis and Western blot analysis

Cellular lysates were prepared as previously described [14]. Lysate aliquots containing 25–50 μg protein were loaded on 10% or 12% SDS–polyacrylamide gels, depending on the molecular mass of the protein to be investigated, electrophoresed and then transferred onto nitrocellulose membrane. The membranes were treated with specific primary antibodies listed below, as recommended by the manufacturer. All secondary antibodies conjugated with horseradish peroxidase were obtained from Abcam (1:5000). Western blots were developed with chemiluminescent substrate Luminata Classico (Millipore). The content of specific

Download English Version:

https://daneshyari.com/en/article/1925270

Download Persian Version:

https://daneshyari.com/article/1925270

Daneshyari.com