Available online at www.sciencedirect.com
SCIENCE DIRECT
Journal of Molecular and Cellular Cardiology 39 (2005) 327–334
Journal of Molecular and Cellular Cardiology
www.elsevier.com/locate/yjmcc

Original article

Activation of IKKβ by glucose is necessary and sufficient to impair insulin signaling and nitric oxide production in endothelial cells

Francis Kim [a,*], Kelly A. Tysseling [a], Julie Rice [b], Byron Gallis [a], Lutfiyah Haji [a], Cecilia M. Giachelli [b], Elaine W. Raines [c], Marshall A. Corson [a], Michael W. Schwartz [a]

[a] *Department of Medicine, Harborview Medical Center, University of Washington, Box 359748, Seattle, WA 98104, USA*
[b] *Department of Bioengineering, University of Washington, Seattle, WA 98195, USA*
[c] *Department of Pathology, University of Washington, Seattle, WA 98195, USA*

Received 22 December 2004; received in revised form 13 April 2005; accepted 16 May 2005

Available online 22 June 2005

Abstract

Hyperglycemic impairment of nitric oxide (NO) production by endothelial cells is implicated in the effect of diabetes to increase cardiovascular disease risk, but the molecular basis for this effect is unknown. In skeletal muscle, diabetes induces activation of inhibitor kappaB kinase (IKKβ), a key cellular mediator of the response to inflammatory stimuli, and this impairs insulin signal transduction via the insulin receptor substrate-phosphatidylinositol 3-OH kinase (IRS-1/PI3-kinase) pathway. Since activation of endothelial nitric oxide synthase (eNOS) is dependent on IRS-1/PI3-kinase signaling, we hypothesized that activation of IKKβ may contribute to the effect of glucose to impair NO production. Here, we show that exposure of bovine aortic endothelial cells to high glucose (25 mM) for 24 h impaired insulin-mediated tyrosine phosphorylation of IRS-1, serine phosphorylation of Akt, activation of eNOS, and production of NO. High glucose treatment also activated IKKβ, and pretreatment with aspirin, a pharmacological inhibitor of IKKβ, prevented both glucose-induced IKKβ activation and the effect of high glucose to impair insulin-mediated NO production. These adverse responses to glucose were also blocked by selective inhibition of IKKβ signaling via overexpression of a kinase-inactive form of the enzyme. Conversely, overexpression of wild-type IKKβ recapitulated the deleterious effect of high glucose on insulin-mediated activation of eNOS. These data demonstrate that activation of IKKβ plays a critical and novel role to mediate the deleterious effects of high glucose on endothelial cell function.
© 2005 Elsevier Ltd. All rights reserved.

Keywords: Nitric oxide; eNOS; Glucose; IKKβ; Endothelial cells

1. Introduction

Insulin-resistant states such as obesity and diabetes are associated with endothelial dysfunction that, in turn, contributes to the increased risk of vascular disease associated with these conditions. The molecular mechanism linking insulin resistance and endothelial dysfunction is not known. Endothelial cells express insulin receptors and, like other insulin-responsive tissues, insulin activates the insulin receptor substrate-1/phosphatidylinositol 3-kinase (IRS-1/PI3-kinase) pathway in these cells, suggesting that endothelial cells are targets for the action of insulin [1,2]. Unlike other insulin-responsive cell types, however, a prominent feature of the response of endothelial cells to insulin is the activation of endothelial nitric oxide synthase (eNOS) and consequent increase of NO production. Since conditions that impair endothelial NO production, including diabetes, obesity and insulin resistance, can promote atherosclerosis, efforts to clarify the cellular mechanisms responsible for endothelial dysfunction in these conditions are a high priority.

Following insulin binding, the insulin receptor undergoes tyrosine autophosphorylation, which leads to tyrosine phosphorylation of IRS-1 and subsequent activation of PI3-kinase. The resultant generation of phosphatidylinosital triphosphate (PIP-3) [3], ultimately leads to activation of several serine kinases including Akt and subsequently, of eNOS by phosphorylation of ser1179 [4]. Since IRS-1/PI3-kinase signaling appears to be required for activation of eNOS by insulin or by shear stress [5], impaired signaling via this pathway

* Corresponding author. Tel.: +1 206 731 8712.
E-mail address: fkim@u.washington.edu (F. Kim).

0022-2828/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.yjmcc.2005.05.009

is hypothesized to induce a global impairment of NO production in endothelial cells. Based on these considerations, we hypothesized that reduced IRS-1/PI3-kinase signal transduction plays a prominent role to mediate the deleterious effects of diabetes on endothelial cell function.

In skeletal muscle, activation of inhibitor kappaB kinase (IKKβ), a key cellular mediator of the response to inflammatory stimuli, is associated with impaired IRS-1/PI3-kinase signaling and is implicated in the pathogenesis of insulin resistance. Specifically, diabetes-induced increases in the levels of free fatty acids (FFA) and tumor necrosis factor (TNF-α) have been shown to increase serine phosphorylation of IRS-1 in skeletal muscle via the activation of IKKβ [6,7]. This in turn impairs insulin-dependent tyrosine phosphorylation of IRS-1 and PI3-kinase activation, and hence attenuates downstream cellular responses to insulin. Combined with evidence that pharmacological inhibition of IKKβ by salicylates improves insulin sensitivity in rodent models of diabetes, activation of IKKβ appears to constitute a critical link between cellular inflammatory pathways and insulin resistance.

In the current work, we investigated the hypothesis that high glucose levels impair insulin signal transduction in endothelial cells via a mechanism involving IKKβ activation. Here we show that after incubation for 24 h in media containing 25 mM of glucose, IKKβ activity is increased in bovine aortic endothelial cells (BAEC) and that this effect is associated with impaired insulin stimulation of both IRS-1/PI3-kinase and NO production. Our finding that pretreatment with aspirin, a pharmacologic inhibitor of IKKβ, reverses this impairment of insulin-dependent NO production implicates IKKβ as a mediator of this response. More direct evidence in support of this conclusion stems from our finding that expressing a dominant negative construct of IKKβ protects BAEC from the deleterious effects of high glucose, while overexpression of a wild-type IKKβ recapitulates the effects of high glucose on signaling via IRS-1/pAkt/peNOS. Taken together, these findings support the hypothesis that activation of IKKβ mediates the negative effects of high glucose on insulin signal transduction and NO production by endothelial cells.

2. Materials and methods

2.1. Materials

Anti-eNOS monoclonal antibody (H32) was obtained from BIOMOL Research Laboratories (Plymouth Meeting, MA), while anti-phospho-eNOS (Ser1177), anti-IRS-1, phospho-Akt (Ser473), anti-Akt rabbit polyclonal antibodies and anti-phosphotyrosine monoclonal antibody were obtained from Cell Signaling (Beverly, MA). Anti-IKKβ antibody (H-470) and IKK substrate (GST-IκBα) were purchased from Santa Cruz (Santa Cruz, CA), aspirin and DAF-2 DA were purchased from Alexis Biochemicals (San Diego, CA) and regular human insulin (Humulin) was purchased from Eli Lilly Inc. (Indianapolis, IN). Endothelial basal medium (EBM) was purchased from Clonetics (Walkersville, MD) and Dulbecco's modified Eagle's medium (DMEM) was obtained from Biowhittaker (Rockland, ME).

2.2. Cell culture

BAEC were grown in DMEM in 10% fetal bovine serum and subsequently incubated in low (5 mM) or high (25 mM) glucose for 24 h. Prior to initiation of experiments, BAEC were serum-starved in DMEM containing 0.1% fetal bovine serum overnight and then for 3 h in EBM (Clonetics, Walkerville, MD). During the starvation period, DMEM or EBM was supplemented with glucose to continuously maintain concentrations of 5 or 25 mM glucose. In some experiments, BAEC were incubated with aspirin (1 mM) or vehicle overnight prior to stimulation with insulin (100 nM) or vehicle.

2.3. Cell lysis and immunoprecipitation

All immunoprecipitations and Western blots were performed as described [4], using equal amounts of total protein for each condition and experiment. SDS gel electrophoresis was performed using either a 4% by 20% gradient gel or 9% gel.

2.4. Measurement of nitric oxide production

NO content was measured using a modification of established procedures [8]. BAEC were grown to 80% confluency on coverslips coated with 2% gelatin and serum-starved for 3 h in the dark in phenol-red-free EBM. Cells were then incubated in 4,5-diaminofluorescein diacetate (DAF-2 DA; 10 μM) for 20 min, washed twice with ice-cold PBS (in the dark), fixed with 2% paraformaldehyde (Electron Microscopy Sciences, Fort Washington, PA) for 3 min and again washed twice with cold PBS. Cover slips were then mounted onto slides with Gel Mount (Sigma, St. Louis, MO) for analysis by fluorescence microscopy. NO production was measured by fluorescence quantification using an Olympus fluorescence microscope with a FITC filter (excitation 465–495 nm, emission 515–555 nm) and a Spot digital camera (Diagnostic Images, Inc., Sterling Heights, MI) to capture images. All images were taken from the same area of each slide during the first 30 s of light exposure to avoid fluorescence decay. Fluorescence intensity of the images was quantified using Image J software (National Institutes of Health).

2.5. IKK activity assay

As previously described [9], BAEC were lyzed in buffer containing phosphatase and protease inhibitors [4], immunoprecipitated with 4 μg of rabbit polyclonal anti-IKKβ antibody (SC-7607) and protein A sepharose for 3 h and washed four times in lysis buffer and twice in protein kinase buffer [9]. Resultant immune pellets were incubated in 50 μl of kinase assay buffer containing 2 μg of GST-IκBα, 25 μCi (γ

Download English Version:

https://daneshyari.com/en/article/10954355

Download Persian Version:

https://daneshyari.com/article/10954355

Daneshyari.com