High glucose increases glomerular filtration barrier permeability through activation of protein kinase G type Iα subunits

A. Piwkowska[1], D. Rogacka[1], M. Kasztan[2], S.Angielski[1], M. Jankowski[1,2]

[1]Mossakowski Medical Research Centre Polish Academy of Sciences, Laboratory of Molecular and Cellular Nephrology, Gdańsk, Poland
[2]Medical University of Gdańsk, Department of Therapy Monitoring and Pharmacogenetics, Poland.

INTRODUCTION AND OBJECTIVES

The glomerular filtration barrier is responsible for utrafiltration of plasma in the renal glomeruli. This barrier comprises an innermost fenestrated endothelium, the glomerular basement membrane, and a podocyte cell layer that faces the urinary space. The extended primary processes of these highly specialized epithelial cells form foot processes that interdigitate with processes of neighboring podocytes to form a filtration slit bridged by the slit diaphragm. The glomerular filtrate passes through the endothelial fenestrae, the basement membrane, and finally through the slit diaphragm, which acts as a molecular sieve [1].
The increase in the permeability of the glomerular barrier filtration to albumin is a well-known feature of diabetic microvasculature and a negative prognostic factor for vascular complications. However, the underlying mechanisms are incompletely understood. We demonstrated recently that superoxide anion generation increases dimerization of protein kinase G type Iα (PKGIα) subunits, leading to podocyte dysfunction [2]. Here we investigated whether high glucose is involved in PKGI-dependent hyperpermeability of the glomerular filtration barrier.

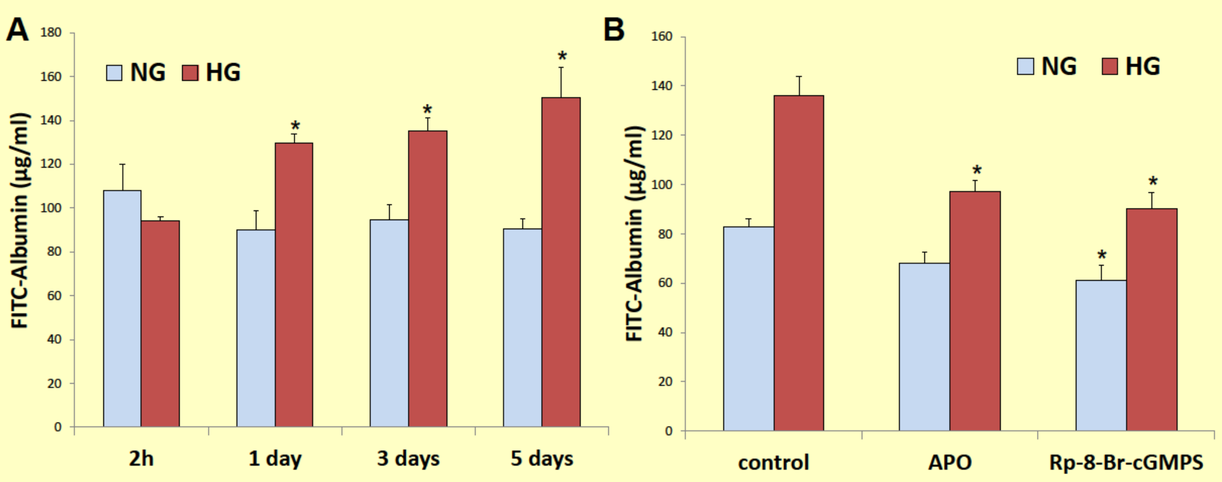
METHODS

Preparation and culture of rat podocytes **All experiments were approved by the local ethics committee (No. 5/2011). Female Wistar rats weighing 100–120 g were anesthetized with thiopental (70 mg per kg body weight, i.p.) The kidneys were excised and minced with a scalpel and then pressed through a system of sieves with decreasing pore diameters (160, 106, and 53 mm) to obtain a suspension of glomeruli in RPMI 1640 supplemented with 10% FBS, 100 U/ml penicillin, and 100 mg/ml streptomycin. The final suspension of glomeruli was plated in type I collagen-coated culture flasks and maintained at 37°C in an atmosphere of 95% air/5% CO_2 for 5–7 days. The outgrowing podocytes were trypsinized and passed through sieves with 33-mm pores to remove the remaining glomerular cores. The suspension of podocytes was seeded in culture flasks and cultivated at 37°C in an atmosphere of 95% air/5% CO_2. Experiments were performed using podocytes cultivated for 12–20 days. Podocytes for the different experiments were cultured in media with either normal (NG, 5.6 mM) or high (HG, 30 mM) glucose for 5 days. As an osmotic control, was used L-glucose.**

Western blot analysis **In the conventional Western blot procedure, podocytes were washed with ice-cold PBS and homogenized in ice-cold lysis buffer, on ice. Lysates were remain on ice for 30 min, then centrifuged for 20 min at 15000×g. Equal amounts of protein extract were resolved by SDS-PAGE and analyzed by immunoblotting. The protein bands were detected with the colorimetric 5-bromo-4-chloro-3-indolylphasphate/nitroblue tetrazolium (BCIP/NBT) system. To obtain non-reducing conditions, we used maleimide (100 mM) in the homogenization and lysis buffers to alkylate thiols and prevent thiol disulfide exchange.**

Permeability assay **Transepithelial permeability to albumin was evaluated by measuring the diffusion of FITC-labeled BSA across the podocyte monolayer as described by Oshima *et al.* [3] with minor modifications [4].**

RESULTS



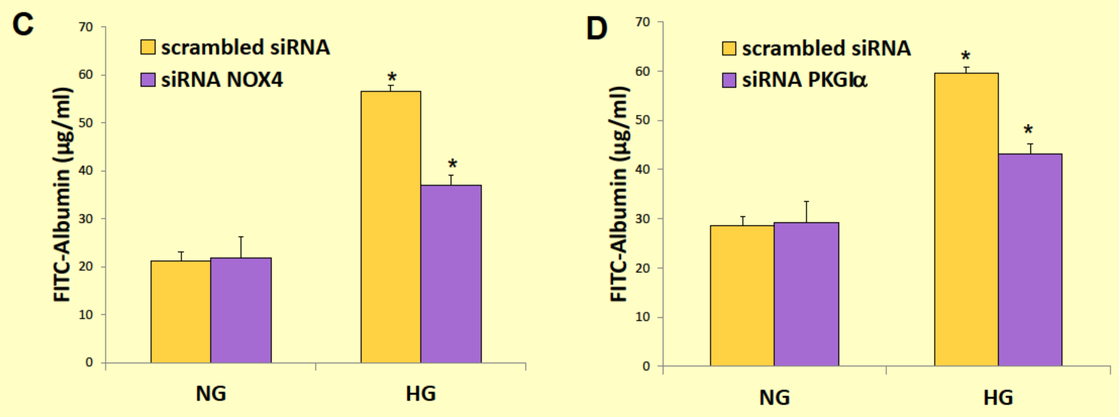


Figure 1. High glucose increases permeability to albumin across a podocyte monolayer via ROS-dependent PKGI activation.
(A) The effect of high glucose concentration on podocyte permeability to albumin. Rat podocytes were exposed to either NG (5.6 mM) or HG (30 mM) for the indicated time periods. (B) Cultured rat podocytes were incubated with NG and HG for 5 days with or without the NAD(P)H oxidase inhibitor apocynin (APO; 50 µM) and the PKG inhibitor Rp-8-Br-cGMPS (50 µM). Results from four to six experiments are shown as means ± SEM. *P<0.05 compared to untreated podocytes (B). The effect of downregulation of NOX4 (C) or PKGIα (D) on high glucose-evoked podocyte permeability to albumin. The values shown represent the mean ± SEM of four independent experiments. *P<0.05 compared to non-transfected podocytes .

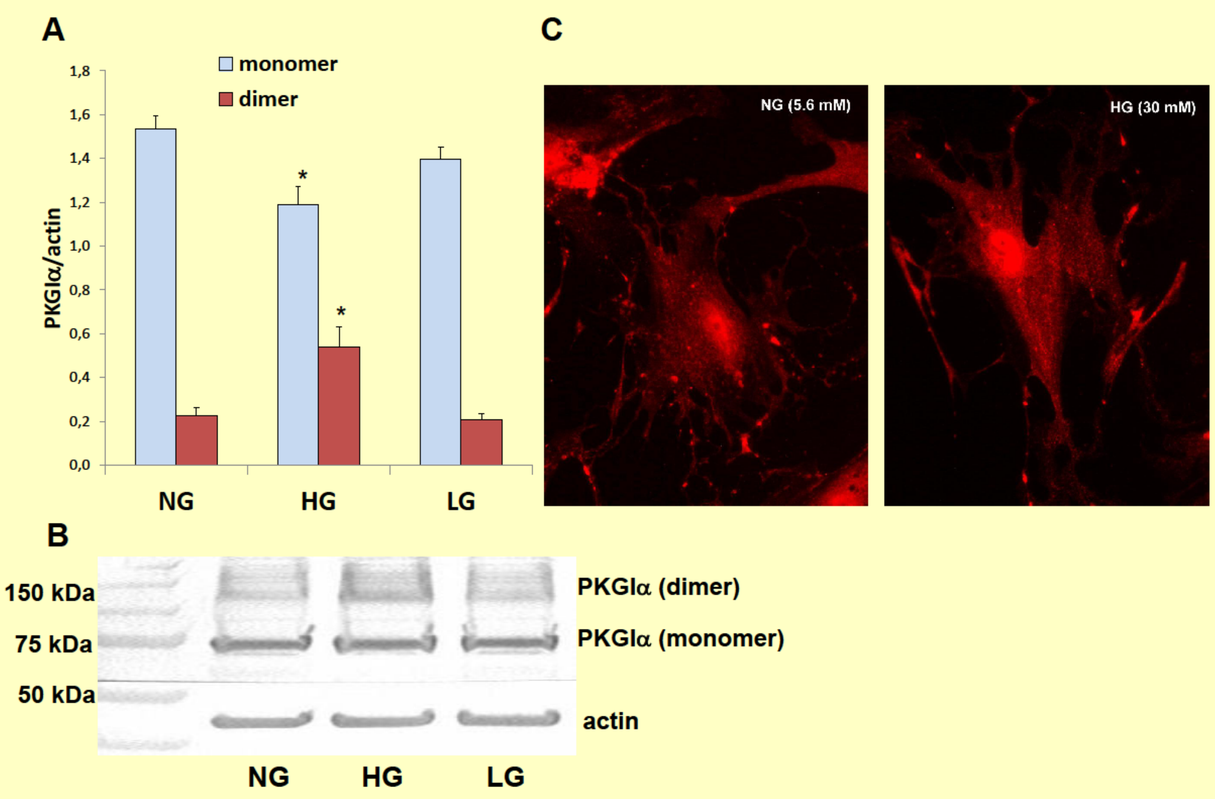


Figure 2. The effects of high glucose on PKGIα dimerization (A and B) and on the sub-cellular distribution of PKGIα (B) in cultured rat podocytes.
PKGIα interprotein disulfide bond formation in the presence of HG (30 mM) or NG (5.6 mM) is reported as the ratios of the monomer and dimer band intensities to the actin band intensity (A). A representative immunoblot is shown in (B). The values shown represent the mean ± SEM of three independent experiments. *P<0.05 compared to control. The effect of HG and NG on PKGIα distribution (C) was visualized by fluorescence microscope (Olympus IX51).

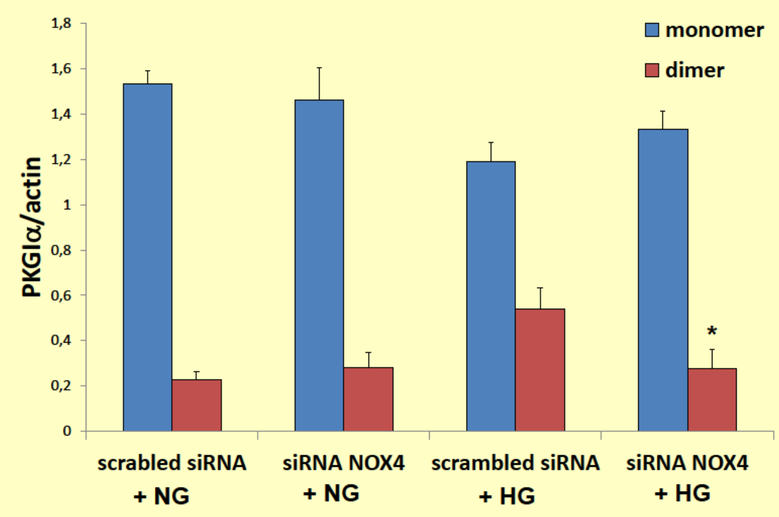


Figure 3. The effect of NOX4 gene silencing on PKGIα dimerization in cultured rat podocytes.
The effect of NOX4 small interfering RNA (siRNA) and scrambled siRNA on PKGIα dimerization. Densitometry of the PKGIα band was normalized to the actin band. The values shown represent the mean ± SEM of four independent experiments. *P<0.05 versus transfection with appropriate scrambled siRNA.

CONCLUSIONS

This study revealed a novel mechanism for high glucose-mediated regulation of filtration barrier permeability and suggested that it plays a role in the development of diabetic glomerular nephropathy. First, high glucose concentration induced an increase in albumin permeability hat was dependent on PKGIα activation. Second, the high glucose-induced increse of albumin permeability requires activation of the NOX4 subunit of NAD(P)H oxidase.

References:
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