HIGH GLUCOSE INDUCES INSULIN RESISTANCE IN RAT CULTURED PODOCYTES VIA AMPK-PTEN PATHWAY

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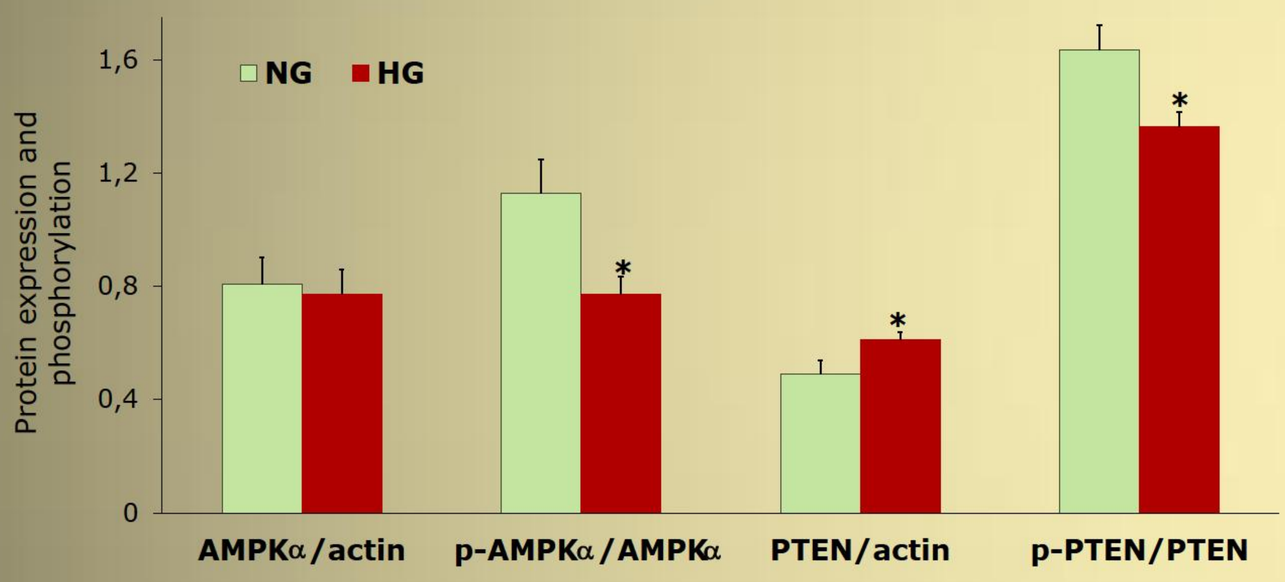
INTRODUCTION AND OBJECTIVES

Diabetic nephropathy (DN) is a chronic progressive disease that affects up to 40% of patients with diabetes mellitus. The early clinical manifestation of DN is microalbuminuria, which can progress to evident proteinuria and renal dysfunction. Glomerular visceral epithelial cells (podocytes), as part of the filtration barrier, play an important role in the development of DN, and their numbers are significantly reduced in both type 1 and type 2 diabetic patients. In addition to the decreased number of podocytes, structural damage to podocytes (podocytopathy) is accompanied by foot process effacement, and it is associated with the progression of proteinuria. In diabetes, podocytes are exposed to elevated glucose levels. Growing evidence indicates that high glucose concentrations affect podocyte metabolism, leading to cell hypertrophy and higher mitochondrial content. Podocytes are direct targets for insulin [1,2], and insulin signaling is essential for normal glomerular function [3]. One of the proteins involved in the regulation of insulin-dependent glucose uptake is AMP-activated protein kinase (AMPK), which appears to positively regulate insulin signaling. AMPK plays an important role in regulating cellular energy homeostasis, and it functions as an energy sensor to provide metabolic adaptation under conditions of cellular stress, when the ATP-to-AMP ratio decreases. Phosphorylation of Thr^{172}, localized within the kinase domain of the α subunit, is required for enzyme activation [4]. Phosphatase and tensin homologue deleted on chromosome 10 (PTEN), which is a dual-function lipid and protein phosphatase, negatively regulates insulin signaling. PTEN phosphatase activity is regulated by inactivating phosphorylation on PTEN Ser^{380} and $Thr^{382/383}$ [5]. Disturbances in insulin signaling accompanied by insulin resistance can lead to various intracellular events. We hypothesized that high glucose concentrations would lead to disturbances in interactions between AMPK and PTEN proteins in podocytes.

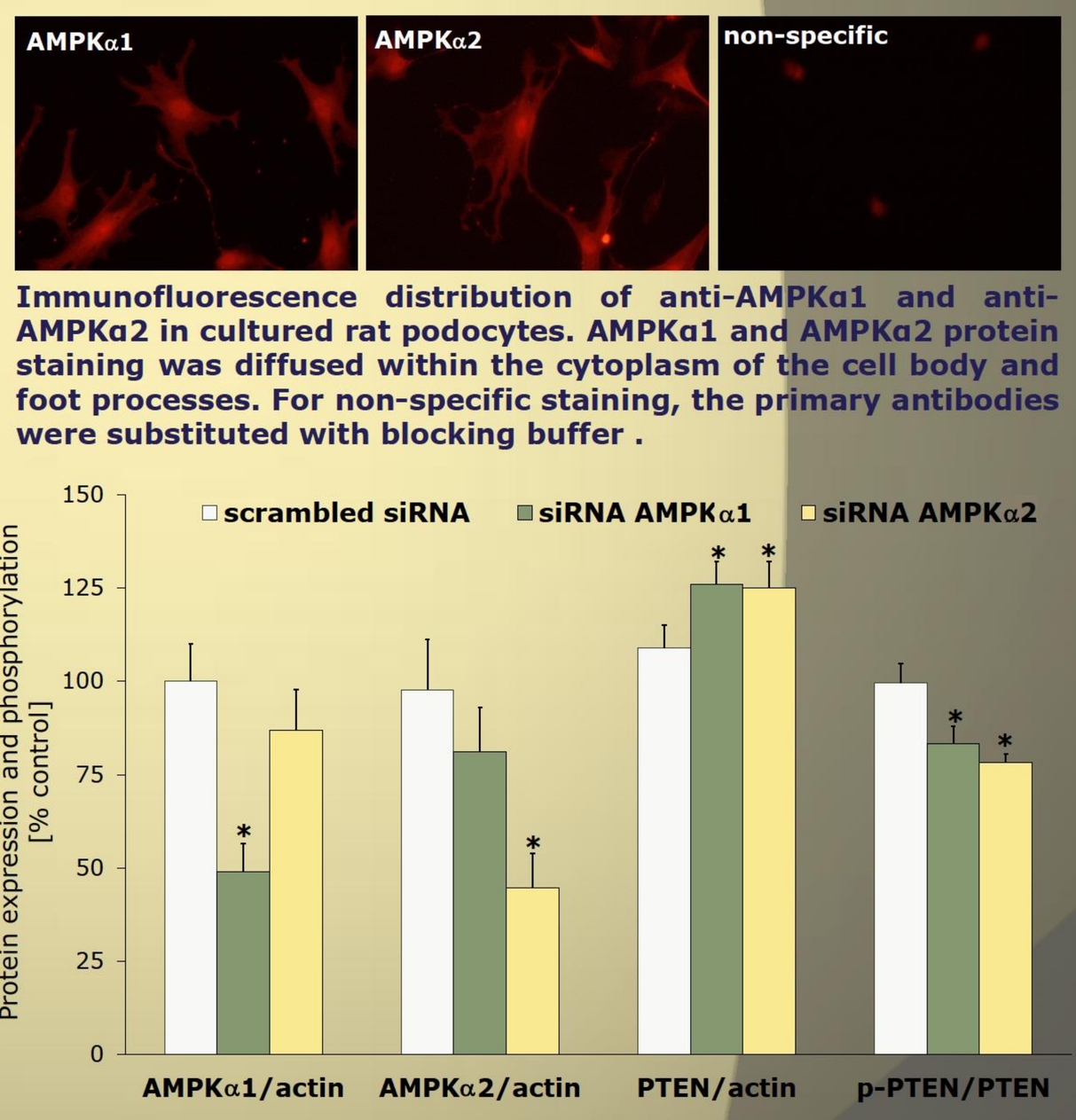
METHODS

Experiments were performed in primary rat podocytes cultured with normal (NG, 5.6 mM) or high (HG, 30 mM) glucose concentrations for 5 d. For the osmotic control, cells were cultured in L-glucose–supplemented NG medium. Glucose uptake was measured by the addition of 1 μCi/well of (1,2-^{3}H)-deoxy-D-glucose diluted in nonradioactive glucose (50 μM final concentration) with or without 300 nM insulin for 3 min. Before glucose uptake experiments cells were incubated for 2 h with serum- and glucose-free RPMI 1640 medium. Immunodetection methods were used to detect AMPK and PTEN proteins, and their phosphorylated forms (anti-p-AMPKα on Thr^{172} and anti-p-PTEN on $Ser^{380}/Thr^{382/383}$). Isoforms of AMPK were detected by RT-PCR. AMPK activity was modified by siRNA for AMPK isoforms α1 and α2. After transfection, cells were cultivated in NG medium for 5 d. Gene silencing was monitored at the protein level by western blotting.

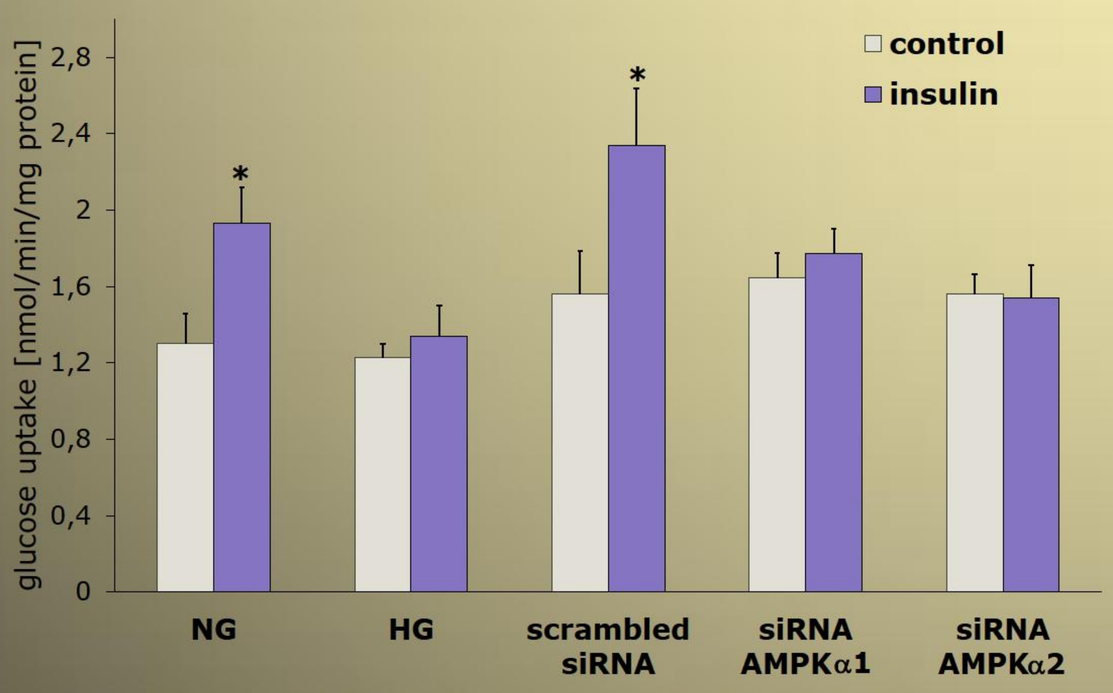
RESULTS



High glucose concentration decreased the phosphorylation of AMPK in podocytes without any significant changes in AMPK protein levels. PTEN protein levels increased, whereas PTEN phosphorylation decreased in HG medium–cultured podocytes compared to cells cultured in NG medium.



Immunofluorescence distribution of anti-AMPKα1 and anti-AMPKα2 in cultured rat podocytes. AMPKα1 and AMPKα2 protein staining was diffused within the cytoplasm of the cell body and foot processes. For non-specific staining, the primary antibodies were substituted with blocking buffer .



In NG medium–cultured podocytes, insulin-stimulated glucose uptake increased, whereas it was abolished after incubation of cells in HG medium. Knockdown of AMPKα1 or AMPKα2 protein expression abolished the insulin-dependent increase in glucose uptake into podocytes cultured in NG medium, but had no significant effect on basal glucose transport into these cells.

Knockdown of AMPKα1 or AMPKα2 protein expression resulted in increases in the PTEN protein level in podocytes cultured in the presence of normal glucose concentration, while PTEN phosphorylation was decreased. Scrambled siRNA had no effect on either PTEN protein levels or phosphorylation in podocytes.

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CONCLUSIONS

We found that impairment of insulin induction of glucose uptake into podocytes cultivated in the presence of high glucose concentrations for long periods of time is associated with increased PTEN levels and decreased PTEN phosphorylation in an AMPK-dependent manner.