The influence of diabetic environment on phosphate homeostasis in podocytes

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INTRODUCTION

Inorganic phosphate (Pi) and pyrophosphate (PPi) are two key substances in a calcification process. Pi is one of the building blocks of hydroxyapatite and also a mineralization promoter, while PPi is the strongest inhibitory factor in this process. Pathological calcification of soft tissues may occur in the final stage of chronic kidney disease caused by diabetic nephropathy. The hyperphosphatemia present at that time contributes to the dysfunction of kidneys, in particular a glomerular filtration barrier. An important element of this barrier are foot processes of podocytes – glomerular epithelial cells. PPi can be a protective factor for podocytes by controlling the phosphate balance in the glomerular filtrate and preventing excessive formation of hydroxyapatite.

The aim of the study is to investigate the influence of the diabetic environment on phosphate homeostasis in podocytes and how it affects the function of these cells.

MATERIALS AND METHODS

Conditionally immortalized human podocytes were cultured in medium with standard (SG – 11 mM) and high glucose concetration (HG – 30 mM) for five days.

We measured the activity of the two main enzymes producing intracellular Pi and PPi – tissue nonspecific alkaline phosphatase (TNAP) and ectonucleotide pyrophosphatase/phosphodiesterase 1 (eNPP1), respectively.

The amount of type III sodium-dependent phosphate transporter PiT 1 was examined by real-time PCR and western blot analysis. This protein is responsible for the intracellular influx of phosphorus ions. Additionally, the cellular localization of the PiT 1 transporter was investigated using fluorescence microscopy.

RESULTS

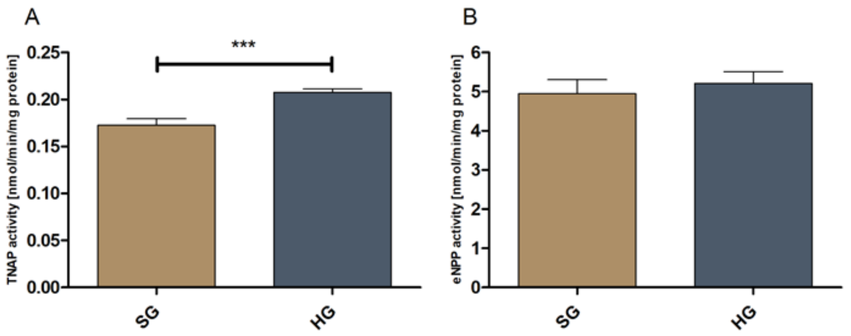

Figure 1. Activity of TNAP and eNPP1 enzymes in cultured podocytes.
(A) In HG conditions, TNAP activity increases resulting in elevated Pi generation (p = 0.001, n = 7). (B) In contrast, the production of PPi by eNPP1 does not change in HG (n = 9).

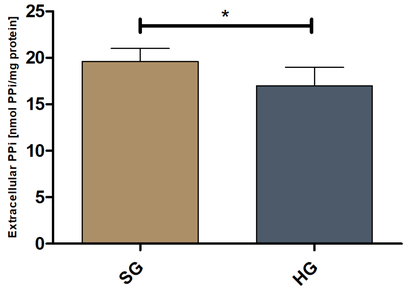

Figure 2. Amount of extracellular PPi in cultured podocytes.
Podocytes were grown in PBS supplemented with an appropriate amount of glucose for 2 hours. The concetration of PPi in the PBS solution was then determined as extracellular PPi, which was related to the amount of protein. Under HG conditions, there is a significant decrease in the extracellular amount of PPi (p = 0.026, n = 7).

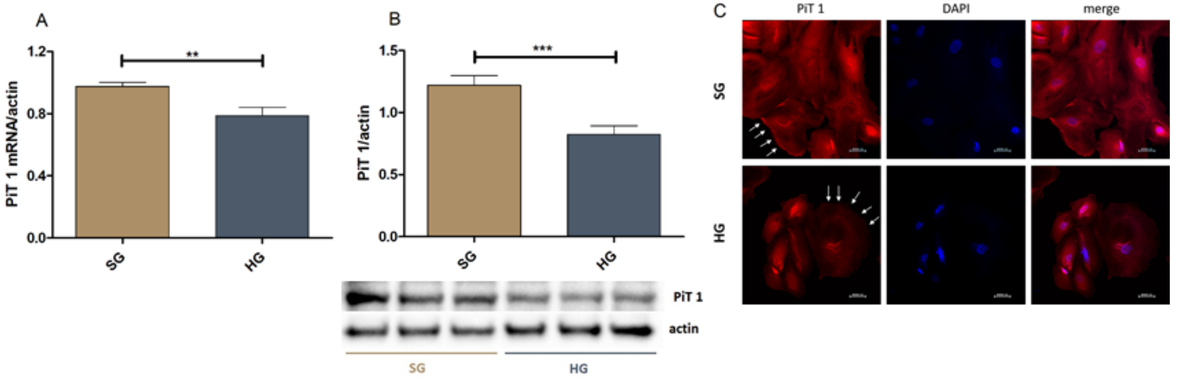

Figure 3. The amount and cellular localization of the sodium-dependent phosphate transporter PiT 1.
(A) Real-time PCR showed a decrease in the amount of PiT 1 mRNA in podocytes cultured in HG (p = 0.006, n = 9). (B) Similar results were obtained for the PiT 1 protein in the western blot analysis (p = 0.0009, n = 13). (C) In the microscopic examination, a decrease in fluorescence intensity was observed in podocytes cultured under HG conditions. In addition, the exposure of the PiT 1 transporter in the plasma membrane was significantly reduced.

CONCLUSIONS

The above data suggest that the high glucose conditions, observed in diabetes, increase the production of Pi by the podocyte. Moreover, a decrease in the amount of PPi in the extracellular environment was noticed. Observed changes in Pi/PPi balance could favor a calcification processes in podocytes.

ACKNOWLEDGEMENTS

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