

High D-glucose reduces promoter activity of human equilibrative nucleoside transporter 1 in human umbilical vein endothelium

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Abstract

Reduction of adenosine uptake by human equilibrative membrane transporters 1 (hENT1) in human umbilical vein endothelial cells (HUVEC) from gestational diabetes, or in HUVEC from normal pregnancies exposed to high extracellular D-glucose, is associated with reduced hENT1 mRNA expression. We studied the effect of high D-glucose on the transcriptional activity of the promoter region of SLC29A1 gene (for hENT1) in HUVEC. Methods: Cells were isolated and cultured in medium 199 (Ethics committee approval and informed patient consent were obtained). Fragments of SLC29A1 promoter (-3100, -2056, -1016 and -697 bp from ATG) were subcloned in pGL3 vector, upstream firefly luciferase reporter gene. Cells were co-transfected with hENT1-promoter constructs and pRL-TK vector by electroporation (320 V, 20 ms) and exposed to 5 or 25 mM D-glucose (24 hrs). Results: firefly/renilla luciferase activity was similar in all constructs transfected in 5 mM D-glucose. However, 25 mM D-glucose was associated with reduced transcriptional activity of sequences -697 to -1016 bp and -2056 to -3100 bp. Conclusions: These results suggest that the reduced hENT1 mRNA level detected in HUVEC exposed to high D-glucose could result from altered transcriptional activity of SLC29A1 promoter, most likely related to activation of repressor sequences of this gene.