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Effect of High Glucose on Fructose Uptake in Renal Proximal Tubule Cells

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Background: Fructose is a nutrient as well as a potent agent for formation of advanced end product in diabetes. And abnormal fructose handling in the proximal tubule may play an important role in the development of diabetic nephropathy. Thus, we investigated effect of high glucose on fructose uptake and its related signaling pathway in the primary cultured rabbit renal proximal tubule cells (PTCs).

Methods: The PTCs were grown in D-MEM/F-12 medium with 15 mM HEPES and 20 mM sodium bicarbonate (pH 7.4). Immediately prior to use of the medium, three growth supplements (5 μg/mL insulin, 5 μg/mL tranferrin, and 5 10-5 M hydrocortisone) were added. Fructose uptake, PKC/cAMP assay, and Western blotting were performed.

Results: When PTCs were incubated with 25 mM glucose ($\geq$12 hr) significantly inhibited fructose uptake compared to control, while 25 mM mannitol or L-glucose did not affect. In Western blotting, high glucose decreased the GLUT5 protein level. High glucose-induced inhibition of fructose uptake was blocked by cytochalasin B (GLUT blocker). SQ 22536, adenylate cyclase inhibitor or myristoylated amide 14-22, protein kinase A (PKA) inhibitors and PTX, an agent the catalyzes ADP-ribosylation of the α subunits of the heterotrimeric guanine nucleotide regulatory proteins Gi, Go, and Gt, prevented high glucose-induced inhibition of fructose uptake. In addition, high glucose-induced inhibition of fructose uptake was blocked by neomycin or U 73122, phospholipase C (PLC) inhibitors, and staurosporin or bisindolymaleimide I, protein kinase C (PKC) inhibitors. When OAG or TPA, PKC activators, was added to the PTCs, they significantly down-regulated fructose uptake compared to control. High glucose-induced inhibition of fructose uptake was blocked by mepacrine, AACOCF3, phospholipase A2 (PLA2) inhibitors. We also examined whether the MAPK pathway is involved in high glucose-induced inhibition of fructose uptake. PD 98059, a p44/42 mitogen activated protein kinase (MAPK) inhibitor or SB 203580, a p38 MAPK inhibitor was treated to the PTCs. PD 98059, but not by SB 203580, prevented high glucose-induced inhibition of fructose uptake.

Conclusion: High glucose inhibits fructose uptake via both PKA, PLC/PKC, PLA2, and MAPK signal pathways in the PTCs