Effect of ATP on Na+/glucose Cotransporter Activity in Renal Proximal Tubule Cells

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Background : Extracellular ATP plays an important signaling role in the regulation of renal function. However, the effect of ATP on Na/glucose cotransporter in proximal tubule cells was not elucidated. Therefore, this study was performed to examine the action of ATP on Na/glucose cotransporter and its related signal pathways in primary cultured rabbit renal proximal tubule cells (PTCs).

Methods : Male New Zealand White rabbits (1.5-2.0 kg) were used for these experiments. PTCs were grown in hormonally defined serum free D-MEM/F-12 media. a-MG uptake, cAMP assay, and western blotting analysis were performed.

Results : ATP increased a-MG uptake in a time (>15 min) and dose (>10-6 M) dependent manner. In this study, 10-4 M ATP for 6 hr was treated to the PTCs. In the experiment of kinetic properties, ATP increased a-MG uptake by the increase of Vmax (control : 0.49±0.02 vs. ATP : 1.34±0.02 nmol/mg protein/min, $p<0.05$), without affecting the Km (control : 1.63 ± 0.01 vs. ATP: 1.61±0.02 mM, p=NS). ATP-induced increase of a-MG uptake was blocked by actinomycin D (an inhibitor of gene trasnscription) and cycloheximide (an inhibitor of protein synthesis), suggesting the new protein synthesis. ATP-induced stimulation of a-MG uptake was correlated with the increase of both SGLT1 and SGLT2 expression levels. ATP-induced stimulation of a-MG uptake was blocked by pertussis toxin (PTX, Gi protein inhibitor), SQ22536 (adenylate cyclase inhibitor), and protein kinase A (PKA) inhibitor amide 14/22 (PKI). ATP also increased cAMP formation. This result suggests that cAMP/PKA pathway is involved in the action of ATP. In addition, ATP-induced stimulation of a-MG uptake was blocked by SB 203580 (p38 kinase inhibitor), but not by PD 98059 (p42/44 MAPK kinase inhibitor) and SP 600125 (JNK inhibitor). Indeed, ATP induced phosphorylation of p38 MAPK.

Conclusion : ATP increases Na/glucose cotransporter activity via cAMP and p38 MAPK in renal proximal tubule cells.