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Regulatory Mechanism of Expression and Magnesium Transport of Paracellin-1, a Novel Magnesium Transporter, in Renal Tubular Cells (Phosphorylation of Paracellin-1 by Protein Kinase A and Magnesium Transport)

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Summary

The magnesium balance of whole body is regulated by the kidney which adapts magnesium excretion based on net magnesium absorption from intestine. Renal magnesium filtrated in the glomeruli is predominantly reabsorbed through the paracellular pathway in the thick ascending limb of Henle. Paracellin-1 belongs to the claudin family of tight junction (TJ) proteins and possibly plays a critical role in the reabsorption of magnesium. So far, we reported that the phosphoserine level of paracellin-1 in hypertensive rats is lower than that in normotensive rats and urinary magnesium excretion is increased in hypertensive rats. In the present study, we examined the regulatory mechanisms of phosphorylation of paracellin-1 and the effect of phosphorylation on magnesium transport.

Paracellin-1 was stably expressed in Madin-Darby canine kidney (MDCK) cells using a Tet-OFF system. The phosphorylation of paracellin-1 is upregulated by fetal calf serum (FCS). This phosphorylation was completely inhibited by H-89, a PKA inhibitor. Without FCS, dibutyryl cAMP (DBcAMP) increased the phosphoserine level of paracellin-1 in a concentration-dependent manner. The phosphorylated paracellin-1 elicited increases of transepithelial electrical resistance and transepithelial transport of Mg^{2+}. Vasodilator-stimulated phosphoprotein (VASP) was also phosphorylated in the presence of FCS or DBcAMP. PKA was immunoprecipitated with paracellin-1, but VASP was not. In cells expressing a dephosphorylated mutant (Ser160Ala) of VASP, paracellin-1 was phosphorylated by DBcAMP and was associated with ZO-1, a tight junctional scaffolding protein, without integral cell-cell junctions. We suggest that PKA directly phosphorylates paracellin-1, resulting in the localization to TJ and the maintenance of Mg^{2+} reabsorption.

In conclusion, we found that paracellin-1 is directly phosphorylated by PKA and the phosphorylated pracellin-1 is distributed at TJ. In hypertensive rats, the reduction of phosphorylation level of paracellin-1 may cause increase in urinary magnesium excretion.