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Functional changes of paracellin-1, a magnesium transporter, by salt intake and clarification of regulatory mechanisms of paracellin-1 (Effect of phosphorylation on magnesium transport by paracellin-1)

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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 urinary magnesium excretion was increased in hypertensive rats compared with normotensive rats. Although the paracellin-1 expression level in hypertensive rat was not different from that in normotensive rat, the phosphoserine level of paracellin-1 in hypertensive rat was lower than that in normotensive rat. In the present study, we examined the regulatory mechanisms of phosphorylation of paracellin-1. Furthermore, we investigated the roles of phosphorylation in magnesium transport.

Rat paracellin-1 inserted into FLAG-tagged vector was transfected into MDCK cells. Paracellin-1 was localized at TJ and increased in transepithelial electrical resistance (TER). Furthermore, paracellin-1 increased in the transepithelial magnesium transport from apical to basal sides. Immunoprecipitation showed that paracellin-1 was phosphorylated at serine residue in the non-stimulated conditions. H-89, a protein kinase A (PKA) inhibitor, decreased in the phosphoserine level of paracellin-1. *In vitro* analysis using GST fusion protein revealed that the Ser217Ala mutant was not phosphorylated by PKA. Furthermore, the Ser217Ala mutant showed no increases of TER and the transepithelial magnesium transport in MDCK cells. The Ser217Ala mutant was distributed in lysosome as well as the wild-type pracellin-1 treated with H-89.

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