Involvement of paracellin-1 in salt-sensitive hypertension (Effect of high-salt intake on paracellin-1 expression and establishment of paracellin-1-expressing cells)

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Most magnesium filtered through the glomerular membrane is reabsorbed in the thick ascending limb (TAL) of Henle loop. So far, we have been reported that urinary magnesium concentration in Dahl salt-sensitive rats is higher than that in normal rats. We hypothesized that the decrease of magnesium reabsorption affects crisis and development of hypertension. It is, however, difficult to clarify the mechanisms of urinary magnesium loss because magnesium transporter has not been identified. Recently, Simon *et al.* identified a new protein, named paracellin-1. This protein is expressed in TAL tight junctions and might play a critical role in the control of paracellular permeability for magnesium and calcium. In the present study, we determined whether the expression level of paracellin-1 is changed in Dahl salt-sensitive rats. Furthermore, we established the cells stably expressing paracellin-1 and examined the function of paracellin-1.

The expression level of paracellin-1 protein was examined by western blotting. However, there is no signal, suggesting that the commercial antibody did not react with paracellin-1. Next, paracellin-1 mRNA was semi-quantified by reverse transcriptase-polymerase chain reaction. The mRNA levels were same between salt-sensitive and resistant rats. We hypothesized that the regulatory factors are affected by hypertension. To clarify the regulatory mechanisms of paracellin-1, we made Madin-Darby canine kidney (MDCK) cells expressing rat paracellin-1. Paracellin-1 inserted into FLAG-tagged vector was transfected into MDCK cells. Immunoprecipitation showed that paracellin-1 was associated with ZO-1, a peripheral membrane protein. In immunocytochemistry, paracellin-1 was colocalized with ZO-1 in tight junction. Paracellin-1 expression increased the transepithelial electrical resistance, indicating paracellin-1 enhanced the barrier function. Furthermore, transport of $^{45}Ca^{2+}$ from apical to basal was increased in time-dependent manner. The addition of magnesium into apical side inhibited the transport of $^{45}Ca^{2+}$. These results indicate that paracellin-1 is expressed functionally in tight junction and transports calcium and magnesium competitively.