F-C5

일차배양된 속수질 집합관 상피세포에서 세포외부 pH변화에 따른 제 2형 수분통로단백의 인산화와 세포내 수송의 변화

경북대학교 의학전문대학원

최효정, 권태환

The Effects of Altered Extracellular pH on Phosphorylation (Serine 256) and Intracellular Trafficking of AQP2 in Primary Cultured Rat Kidney Inner Medullary Collecting Duct Cells

Hyo-Jung Choi, Tae-Hwan Kwon

Kyungpook National University School of Medicine

Collecting duct water reabsorption in response to vasopressin is mediated by translocation of intracellular AQP2-bearing vesicles to the apical plasma membrane and regulation of the AQP2 abundance. Phosphorylation at serine 256 in the C-terminus of AQP2 (p-AQP2) is involved in the vasopressin-induced AQP2 trafficking to the plasma membrane. In this study we aimed to examine the effects of altered microenvironment, e.g., extracellular pH, on the phosphorylation (serine 256) and intracellular trafficking of AQP2 in the primary cultured inner medullary collecting duct (IMCD) cells of rat kidney. Freshly prepared IMCD suspension was exposed to pH 6.4, 7.4, or 8.4 for 1 hour. In the absence of dDAVP stimulation, semiquantitative immunoblotting revealed that p-AQP2/AQP2 ratio was significantly increased at pH 8.4, while it was unchanged at pH 6.4 and pH 7.4. In contrast, in the presence of dDAVP stimulation (100 pM, 3 min), p-AQP2/AQP2 ratio was significantly increased when exposed to pH 7.4 or 8.4, whereas it was significantly decreased at pH 6.4. We monitored the effects of varying extracellular pH on intracellular pH in IMCD cells. Changes of extracellular pH caused a time-dependent decrease or increase in intracellular pH. The cells were exposed to extracellular pH of 6.4, 7.4 or 8.4 for 1 hour, and the intracellular pH had further dropped to 6.1, 7.2 and increased to 7.65 respectively at 1 hour. Next, IMCD cells were seeded on transwell culture system with hyperosmolar culture medium (640 mOsm/KgH_2O, pH 7.4) for 5 days, and then the cells were exposed to transepithelial pH gradient for 30 min (pH 6.4 at the apical side vs. pH 7.4 at the basolateral side) in the absence or the presence of dDAVP stimulation (1 nM for 30 min) applied to the basolateral side. Laser scanning confocal microscopy (z-sectioned images) revealed that 30 min exposure to culture medium with lower pH (pH 6.4) substantially reduced the dDAVP-induced AQP2 trafficking to the apical plasma membrane. Moreover, cell surface biotinylation assay demonstrated significantly decreased expression of dDAVP-induced phosphorylation of AQP2 (serine 256) at the cell surface of the cells exposed to lower pH (pH 6.4, 30 min) at the apical side. Taken together, altered extracellular pH per se could exert a profound effect on dDAVP-induced AQP2 phosphorylation and trafficking in the collecting duct principal cells.

Key Words: 세포내 pH, 제2형수분통로단백 인산화, 항이뇨호르몬
Intracellular pH, AQP2 phosphorylation, Vasopressin