S-A8

산도변화에 따른 항이뇨호르몬-유도 제2형 수분통로단백의 세포내 수송변화

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최효정, 권태환

Luminal pH Affects Phosphorylation (Serine 256) and Intracellular Trafficking of AQP2 in Inner Medullary Collecting Duct Cells

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Collecting duct cells are continuously exposed to the changes of microenvironment, e.g, luminal pH. The effects of altered extracellular pH (pHe) on dDAVP-induced phosphorylation (S256, p-AQP2) and apical targeting of AQP2 were examined in rat kidney inner medullary collecting duct (IMCD) cells. Freshly prepared IMCD suspension was exposed to buffer with pH 6.4, 7.4, or 8.4 for 1 h. dDAVP (10-10 M, 3 min)-induced AQP2 phosphorylation was more prominent when tubule suspension was exposed to pH 7.4 and 8.4, compared to pH 6.4. When primary cultured IMCD cells were exposed to pHe 6.4, 7.4 or 8.4 for 1 h, intracellular pH became 6.1, 7.2 and 8.1, respectively. IMCD cells cultured in a transwell chamber were exposed to transepithelial pH gradient for 1 h (pH 6.4, 7.4 or 8.4 at the apical side vs. pH 7.4 at the basolateral side). Laser scanning confocal microscopy and cell surface biotinylation assay revealed that exposure to luminal pH 6.4 for 1 h significantly decreased dDAVP (10-9 M, 15 min, basolateral)-induced apical targeting of AQP2 and surface expression of p-AQP2. Importantly, fluorescence resonance energy transfer (FRET) analysis revealed that dDAVP (10-9 M)-induced increase of PKA activity was significantly attenuated when LLC-PK1 cells were exposed to pHe 6.4, compared to pHe 7.4 and 8.4. In contrast, forskolin (10-7 M)-induced PKA activation was not affected. Moreover, dDAVP-induced increase of intracellular Ca2+ was not affected. Taken together, acidosis is likely to decrease dDAVP-induced phosphorylation and apical targeting of AQP2 in IMCD cells, likely via an inhibition of G-protein-cAMP-PKA action.

Key Words: 항이뇨호르몬, pH, G-protein-cAMP-PKA
Vasopressin, Luminar pH, G-protein-cAMP-PKA