Modulation of DRA Function and Expression by Gut Microbes

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DRA has been shown to functionally couple with NHE3 to produce electroneutral NaCl absorption in the mammalian intestine. Therefore, any alterations in DRA function and/or expression significantly influence the intestinal electrolyte and water absorption. As a part of the ongoing studies in our laboratory examining the modulation of the human intestinal NaCl absorption in health and disease, studies were designed to elucidate whether enteropathogenic *Escherichia coli* (EPEC) induced early diarrhea and whether antidiarrheal characteristics of probiotic bacteria involve modulation of the luminal membrane DRA function as well as its expression in the human intestinal model monolayers. Our aims were to test the hypothesis that EPEC infection decreases Cl^--HCO_3^-(OH^-) exchange activity and DRA expression in human intestinal epithelial cells (IECs) to cause diarrhea; whereas probiotics act as pro-absorptive agents by increasing Cl^--HCO_3^-(OH^-) exchange activity and DRA expression. Caco-2 cells in culture and mouse models were utilized for assessing the effects of EPEC infection and probiotics treatment. The effect of bacteria at 30 min to 3 h, or probiotics culture supernatant for 24 h on DIDS sensitive ^{36}Cl- uptake was determined. Cell surface biotinylation and confocal microscopy were utilized to assess expression of ion transporters. Real-time QPCR was utilized to measure mRNA expression. EPEC infection markedly inhibited the Cl^-/OH^- exchange activity (50-75%) in Caco-2 cells via type-III secretion system (TTSS) of the bacterium and effector molecules EspG and EspG2. EPEC infection reduced surface expression of apical anion exchanger, DRA on the plasma membrane (~70%) in Caco-2 cells and in the mouse colon. Treatment of cells with *Lactobacillus acidophilus (LA)/rhamnosus* (LR) or their culture supernatants stimulated Cl^-/OH^- exchange activity via a PI3 kinase dependent pathway. DRA promoter activity and mRNA expression was also significantly enhanced by LA/LR culture supernatant treatment. Our data demonstrate that EPEC infection of human intestinal epithelial cells inhibits DRA function, which may underlie the pathophysiology of EPEC-induced early diarrhea. In contrast, probiotics exhibit stimulatory effects on DRA that highlight the potential therapeutic roles of probiotics in prevention of diarrhea associated with enteric infections or inflammatory bowel diseases.

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