Spring 5-4-2017

Elucidating internalization mechanism of the Na-K-2Cl cotransporter 1 and its fate in the endocytotic pathway during protein kinase C activation in epithelial cells

Jesse Smallwood
Valparaiso University

Kenneth Bridgmon
Valparaiso University

Kelli Cook
Valparaiso University, kelli.cook@valpo.edu

Natasha Petreska
Valparaiso University

Elucidating internalization mechanism of the Na-K-2Cl cotransporter 1 and its fate in the endocytotic pathway during protein kinase C activation in epithelial cells

Kenneth Bridgmon, Jesse Smallwood*, Chase Jones, Natasa Petreska, Kelli Cook and Patrice G Bouyer. *Equally contributed authors. Valparaiso University.*

Gut clearance (*i.e.,* fluid secretion) is an important mechanism for host defense. Fluid secretion flushes luminal toxins and prevents bacterial attachment to intestinal epithelial cells, which otherwise would harm the host. In the colon, transepithelial chloride fluid secretion drives fluid secretion. The basolateral Na-K-2Cl cotransporter 1 (NKCC1) is the main protein pumping chloride inside the cell for its secretion by apical chloride channels. Previous studies have demonstrated that activation of the protein kinase C (PKC) causes a rapid internalization of NKCC1, thus decreasing chloride secretion. To date, the protein kinase C downstream targets involved in NKCC1 internalization and the fate of NKCC1 in the endocytic pathway is unknown. Using the human colonic crypt cells T84, we demonstrate that T84 cells express α-adducin and Myristoylated, Alanine-Rich C Kinase Substrate, two substrates of the PKC involved in protein internalization in other cells. In presence of phorbol 12-myristate 13-acetate (PMA), an activator of the conventional and novel PKC, we demonstrate that α-adducin is strongly phosphorylated in T84 cells. Next, we hypothesized that upon activation by PKC, α-adducin binds to NKCC1. In T84 cells subjected to PMA, we show that phospho α-adducin co-immunoprecipitates with NKCC1. Next, we used Mardin Darby Canine Kidney (MDCK) cells stably expressing eGFP-NKCC1. In this model, using immunocytochemistry we show that NKCC1 colocalizes with α-adducin at the plasma membrane during PKC activation. Finally, we tested the fate of NKCC1 in the endocytic pathway. In MDCK cells exposed to PMA, we found that NKCC1 colocalizes with LAMP1, a marker of the lysosome. In conclusion, our data suggest that α-adducin participates to NKCC1 internalization during PKC activation and NKCC1 is targeted for degradation.