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Utilizing Chemogenetic Tools to Study Distal Convoluted Tubule Function

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Abstract

Although it is the smallest segment of the nephron, the distal convoluted tubule (DCT) plays an outsized role in electrolyte homeostasis through regulation of several ions, including sodium. In the DCT, sodium balance is primarily determined by the regulation of the sodium-chloride cotransporter (NCC). When phosphorylated, NCC reabsorbs sodium away from the lumen of the nephron. Dephosphorylation of NCC inactivates this reabsorption resulting in increased sodium excretion. A similar effect is leveraged by a common class of anti-hypertensive medications called thiazides, which block NCC activity. Gaining a better understanding of the molecular mechanism by which NCC is regulated holds high clinical relevance to several diseases including hypertension, FHHt, and Gitelman syndrome. To investigate a novel mechanism that may regulate NCC activity, we implemented a cell-specific chemogenetic approach with Designer Receptors Exclusively Activated by Designer Drugs (DREADD) technology. We bred DCT-specific inducible Cre Recombinase mice (NCC-creERT2) to conditional Gq- GPCR coupled (G-protein-coupled receptors) mice (Gq-DREADD) to create DCT-DREADD mice. These mice maintain an intact thiazide response, indicating that the expression of the DREADD does not alter DCT function at baseline. Next, we activated Gq-GPCR signaling in DCT cells by intraperitoneal injection of the DREADD-specific agonist deschloroclozapine (DCZ) causing a more than 80% reduction in NCC phosphorylation. To confirm that DCZ-induced dephosphorylation of NCC alters electrolyte handling within the nephron we measured sodium excretion following DCZ administration in DCT-DREADD mice. We found that DCZ increased sodium excretion (UNaV) by 215% in DCT-DREADD mice compared to controls. To investigate the kinetics of this effect, we performed a timecourse experiment measuring outcomes from 15 minutes to 24 hours. We posit that the chemogenetic activation of DCT leads to a release of intracellular calcium and subsequent activation of the phosphatase required for the dephosphorylation of NCC.