The thiazide-sensitive NaCl cotransporter: a new target for acute regulation of salt and water transport by angiotensin II

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There is no doubt about the clinical importance of the thiazide-sensitive sodium chloride cotransporter (NCC). The NCC exists exclusively in the distal convoluted tubule and is responsible for 5–10% of NaCl reabsorption by the kidney. The efficacy of an inhibitor of the NCC, chlorothiazide, in lowering blood pressure was first demonstrated in 1958 (3), and thiazide diuretics continue to serve as a low-cost and effective drug for the treatment of hypertension in many patients. Genetic mutations in humans and mice that suppress or heighten NCC function cause hypotension or hypertension, respectively (10).

Despite the importance of NCC function in human health and nearly 50 years of clinical application of NCC inhibitors, the mechanisms of NCC action and regulation are only recently being defined. Thiazide diuretics were in clinical use for decades before physiological studies determined that the thiazide receptor was a NaCl cotransporter (8, 9, 14) and autoradiographic studies using tritiated metolazone binding localized the NCC to the distal convoluted tubule (2). The protein structure and gene sequence of the mammalian NCC were described only 13 years ago (11) and provided the tools for immunolocalization and gene expression studies. These not only confirmed that the NCC was located exclusively in the apical region of distal convoluted tubule cells (1, 19, 22) but also allowed examination of the mechanisms of its regulation.

The elegant work of Sandberg et al. (24) reports important new information about NCC regulation. They demonstrate three novel findings: 1) acute regulation of NCC apical plasma membrane expression in an animal model; 2) NCC regulation by intracellular trafficking; 3) effects induced by infusion of the angiotensin-converting enzyme (ACE) inhibitor captopril and ablated by infusion of captopril with angiotensin II (ANG II), thus suggesting direct regulation of NCC function by ANG II.

Sandberg et al. (24) demonstrate more rapid regulation of NCC than has been reported previously. Several investigators have shown changes in NCC abundance in experimental animals chronically fed low- or high-salt diets (17, 23), loaded with ammonium chloride (12), or treated with steroid hormones including mineralocorticoids (7, 13), glucocorticoids (7), and estradiol (30). Recently, With-No-Lysine[K] (WNK) kinases have been found to regulate NCC expression in Xenopus laevis oocytes (34), and mutations of WNK kinases in humans are associated with familial hyperkalemic hypertension (Gordon syndrome) (25, 33) and Gitelman’s syndrome (10). Genetic conditions associated with abnormally high and abnormally low NCC function, respectively. Salmon calcitonin and amylin infusions produce increased tritiated metolazone binding in renal homogenates within hours (4, 5). However, Sandberg et al. (24) observed a significant reduction in apical membrane NCC within 20 min of exposure to captopril.

It is not surprising that such a rapid response occurs by intracellular trafficking of the NCC protein. Subcellular redistribution of other transport proteins and channels, such as aquaporin 2 (18), ENaC subunits (16), pendrin (26, 27), and H+-ATPase (28, 29), alters functional expression of transporters and thus regulates transport in other renal epithelial cell types, often without increasing total cellular expression. Although subcellular redistribution was not detected in previous studies that examined chronic regulation of NCC using immunolocalization (13, 30), WNK kinases appear to regulate NCC function in X. laevis oocytes, at least in part, by regulating NCC subcellular distribution (34), and defective membrane targeting of NCC has been observed in both Gitelman’s (10) and Gordon syndromes (33). Nonetheless, subcellular redistribution of NCC has not been demonstrated previously in native cells.

Finally, the findings of Sandberg et al. (24) suggest that ANG II may directly control distribution of NCC to the apical plasma membrane in distal convoluted tubule cells. A previous study of mice fed a low-salt diet found reduced NCC protein in AT1a receptor knockouts compared with wild-type mice (6), thus demonstrating that normal NCC expression depends on ANG II stimulation. Several studies, both in vivo and in vitro, have shown that ANG II regulates transport or transporter distribution in other renal epithelial cells. ANG II stimulates sodium and bicarbonate uptake in the early distal tubule (32), apical ENaC activity in CCD principal cells (21), chloride uptake via pendrin in mouse CCD (20), and Na/H exchange and Na-HCO3 cotransport in the proximal tubule (15, 31). The evidence that ACE inhibition suppresses NCC-mediated NaCl transport adds important new knowledge about the direct effects of ANG II on renal epithelial transport and the mechanism of ACE inhibition in the control of blood pressure.

This work by Sandberg and colleagues (24) is the outcome of a carefully designed and executed animal model, the finest quality quantitative immunogold electron microscopy, and correlation with membrane fractionation studies. The animal studies were designed and conducted to determine the effects of captopril and captopril plus ANG II infusions without confounding changes in blood pressure or glomerular filtration rate. Although the membrane fractionation studies alone suggest that ANG II controls the subcellular distribution of NCC, microscopic localization of the transporter was warranted. However, immunofluorescence microscopy was inconclusive. Light microscopic methods, including confocal microscopy, are often inadequate to determine the precise subcellular location of proteins or to detect changes in distribution or abundance in subcellular compartments. The quantitative immuno-
gold analysis performed on ultrathin cryosections and reported by Sandberg et al. represents not only exceptional technical expertise but also an exceptional effort and commitment to excellence to produce this work. As a result, these investigators convincingly and elegantly demonstrate a previously unknown renal response to ACE inhibition, that is, rapid internalization of NCC and thus acute suppression of NaCl uptake in the distal convoluted tubule.

REFERENCES


