Regulated endocytosis of NCC

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THE THIAZIDE-SENSITIVE Na⁺-Cl⁻ cotransporter (NCC) in the aldosterone-sensitive distal convoluted tubule (DCT) is responsible for the reabsorption of as much as 10% of filtered Na⁺-Cl⁻ (13). Notably, this may be a significant overestimate of the quantitative contribution of NCC to renal Na⁺-Cl⁻ handling, in light of the recent demonstration that combined activity of the apical Na⁺-dependent Cl⁻-HCO₃⁻ exchanger SLC4A8 and the Na⁺-independent Cl⁻-HCO₃⁻ exchanger SLC26A4 (pendrin) mediates considerable thiazide-sensitive electroneutral Na⁺-Cl⁻ cotransport in the collecting duct (11); the natriuretic response to thiazides is thus the net effect of inhibiting both NCC in the DCT and SLC4A8/SLC26A4 in the collecting duct. Regardless, the clinical importance of NCC-dependent Na⁺-Cl⁻ reabsorption by the DCT is illustrated by the phenotype of Gitelman syndrome (3), caused almost exclusively by loss-of-function mutations in NCC. A gain-in-function of NCC occurs in familial hyperkalemic hypertension (FHHII; also known as pseudohyopaldosteronism type II or Gordon’s syndrome); however, this disorder is caused by mutations not in NCC but in two of the four WNK (With No K/Lysine) kinases, so named for the absence of a conserved catalytic lysine (6, 20). The involvement of NCC in FHHII ultimately led to an enhanced appreciation of its role in K⁺ homeostasis; NCC activity in the DCT indirectly affects the lumen-negative potential that drives K⁺ excretion in the distal nephron, via its effects on the delivery of Na⁺ to downstream principal cells (10).

NCC is regulated by an emerging cast of characters, which includes angiotensin II (26), aldosterone (2), vasopressin (15, 16), the WNK kinases (4, 7, 18, 21), the aldosterone-induced SGK1 kinase (23), and the STE20/SPS1-related proline/ala-nine-rich kinase (SPAK) and oxidative stress-responsive kinase 1 (OSR1) kinases (7, 18, 21, 32). The mineralocorticoid receptor (7), AT₁ angiotensin II receptor (5), V2 receptor (14), WNK1 (33), WNK3 (22), WNK4 (33), and SPAK (31) are all coexpressed with NCC in the DCT. WNK-dependent phosphorylation and activation of SPAK or OSR1 leads to phosphorylation of a cluster of N-terminal threonines in NCC, resulting in the activation of Na⁺-Cl⁻ cotransport (18, 21). However, coexpression of WNK4 with NCC reveals an additional inhibitory influence on NCC, effects which are blocked by FHHII-associated point mutations in the kinase (7). In particular, the inhibitory effects of WNK4 appear to dominate in mouse models with overexpression of wild-type vs. FHHII mutant WNK4 (10). The various mechanistic models for the regulation of NCC by upstream WNK1, WNK4, and the SPAK/OSR1 kinases have recently been reviewed (7); interactions between WNK4 and both WNK3 (34) and SGK1 (23) also contribute to the complexity. Competing, divergent mechanisms can be reconciled by the likelihood that the physiological context determines whether WNK4 will have an activating or inhibitory effect on NCC. For example, the activation of NCC by the AT₁ angiotensin II receptor appears to require the downstream activation of SPAK by WNK4 (4, 24). Changes in circulating and local levels of angiotensin II (4, 24), aldosterone (2), vasopressin (15, 16), and K⁺ (30) are thus expected to have different and often opposing effects on the activity of NCC in the DCT (4, 7, 32).

Regardless of the mechanistic details, trafficking of the NCC protein appears to play a significant role in its regulation. NCC protein is detectable at the plasma membrane and in subapical vesicles within the DCT (17), with a marked predominance of membrane-associated protein in response to treatment with angiotensin II or a low-salt diet (25, 26), i.e., a net trafficking to the plasma membrane. WNK4 coexpression with NCC reduces transporter expression at the membrane of both Xeno-pus laevis oocytes and mammalian cells (4, 7, 32). Recent reports from two separate laboratories have indicated that the kinase activates lysosomal degradation of the transporter protein, rather than inducing dynamin- and clathrin-dependent endocytosis (29, 35). This occurs through effects of WNK4 on the interaction of NCC with the lysosomal-targeting receptor sortilin (35) and the AP-3 adaptor complex (29).

How might NCC be targeted for regulated endocytosis? An answer has begun to emerge from two papers by Ko et al. (8, 9) on the effect of phorbol esters on NCC, the second of which appears in an issue of the American Journal of Physiology-Renal Physiology (9). The phorbol ester O-tetradecanoyl-phorbol-13-acetate (TPA) reduces NCC expression at the plasma membrane of both X. laevis oocytes and a mouse DCT cell line that expresses NCC (8). Pharmacological dissection revealed that TPA did not exert this effect through the activation of protein kinase C. Rather, TPA appeared to inhibit NCC via activation of the Ras-guanyl-releasing protein 1 (RasGRP1), resulting in downstream activation of H-Ras, Raf, MEK1/2, and the ERK1/2 kinases (8). The MEK1/2 inhibitor U0126 thus blocked the effect of TPA on NCC activity, as did small interfering RNA-mediated downregulation of RasGRP1. RasGRP1 silencing reduced the stimulatory effect of TPA on both H-Ras-GTP levels and phosphorylation of ERK1/2 (8).

Ko et al. (9) have significantly extended these findings in their subsequent paper. TPA was shown to internalize NCC protein via a dynamin-dependent mechanism, the first direct demonstration of regulated endocytosis of NCC. Unlike WNK4 (29, 35), TPA did not affect forward trafficking of NCC, in that inhibition of Golgi transport with brefeldin A had no effect on internalization of the transport protein (9). ERK1/2 activation can induce ubiquitination and thus target proteins for endocytosis and/or degradation. Of particular interest, ERK1/2-dependent phosphorylation of the β- and γ-subunits of the
epithelial Na\(^+\) channel (EnaC) facilitates interaction with the ubiquitin ligase Nedd4 (1, 27). Consistent with this cellular physiology, TPA treatment of mDCT cells expressing endogenous NCC and Madin-Darby canine kidney cells expressing epitope-tagged NCC resulted in ubiquitination of the transport protein (9). This ubiquitination of NCC was dependent on RasGRP1 expression. Pharmacological inhibition of ubiquitination with UBEI-41 abrogated the effect of TPA on ubiquitination and endocytosis of NCC.

In summary, the activation of RasGRP1 by TPA stimulates ERK1/2 phosphorylation via activation of H-Ras, Raf, and MEK1/2, resulting in ubiquitination of NCC and endocytosis of the transporter (8, 9). Why is this pharmacological cascade so crucial? First, this is the first published evidence for a pathway that stimulates endocytosis of NCC (9). Second, the activation of RasGRP1 by a phospholipase C-dependent pathway that stimulates endocytosis of NCC (9). Second, the observation that NCC is ubiquitinated by an ERK1/2-dependent mechanism evokes multiple levels and mechanisms through which aldosterone and other mediators might impact regulated endocytosis of NCC (9). For example, ERK1/2 and SGK1 are likely to intercede considerably in the regulation of NCC ubiquitination, as occurs in the regulation of ENaC (1, 27, 28).

This report (9) clearly is an interesting development in the molecular physiology of NCC. Future issues include the identity of the E3 ubiquitin ligase(s) that ubiquitinitates NCC. NEDD-4 is an obvious candidate, given its dramatic role in the ubiquitin-dependent regulation of ENaC; however, NCC lacks the requisite PY motif to interact with Nedd4 (13). Another possible candidate is the E3 ligase “plenty of SH3” (POSH), involved in the ubiquitination and clathrin-dependent endocytosis of Kir1.1 (ROMK) channels (12).

DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the authors.

REFERENCES


