WNK signaling in the distal tubule: an inhibitory cascade regulating salt transport

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WITH-NO-LYSINE KINASE-1 (WNK1) AND WNK4 ARE MEMBERS of the WNK family of protein kinases. These two proteins have recently come under intense scrutiny because mutations in their genes have been identified as the cause of type II pseudohypoaldosteronism (PHAII) (18). Patients with this autosomal dominant disorder have salt-sensitive hypertension, hyperkalemia, renal tubular acidosis, and suppressed plasma renin activity, which have been largely attributed to increased chloride reabsorption in the distal nephron (8, 13, 15). The mutations found in the WNK1 gene are deletions in the first intron that appear to increase abundance of WNK1 mRNA when assayed in leukocytes (18). Thus overexpression of WNK1 likely causes the clinical abnormalities found in PHAII. This is consistent with the finding that WNK1-deficient mice are hypotensive (26).

WNK1 is a very large, multifunctional protein, with a serine-threonine kinase domain close to its NH2 terminal and a large downstream region, including an autoinhibitory domain and coiled-coil domains, that is probably involved in protein-protein interactions (7, 20, 21). Four potential functional roles for WNK1 in the regulation of salt transport have now been identified. First, it has been shown in the Xenopus laevis oocyte expression system that WNK4 reduces surface abundance of the thiazide-sensitive NaCl cotransporter, NCC. WNK1 binds to WNK4 and inhibits this effect, thereby upregulating surface expression of NCC (19, 24, 25). If this also occurs in the distal convoluted tubule, it might potentially explain the phenotype in PHAII. Indeed, recent data demonstrate that NCC trafficking can occur in vivo (12). Second, WNK1 phosphorylates calcium-binding synaptotagmins and regulates vesicle secretion in neurosecretory cells (6). As other tissues, including the kidney (5), have synaptotagmins, WNK1 could potentially regulate other forms of vesicle trafficking, such as trafficking of membrane transport proteins. Third, WNK1 phosphorylates and activates two protein kinases of the STE20 family, SPAK and OSR1 (17). These proteins have been shown to interact with, and regulate, various members of the electroneutral cation-chloride cotransporter family, including NKCC1 and KCC2 (2, 3, 11). Finally, WNK1 activates the epithelial sodium channel, ENaC (9, 22).

However, the situation is not so simple. It turns out that multiple different WNK1 transcripts are generated, including a ubiquitously expressed, full-length form (L) that includes the kinase domain and a short, kidney-specific form (KS) that is transcribed from an alternative fourth exon, 4a, and is identical to the full-length form from exon 5 onward but lacks the entire kinase domain (1, 10). Remarkably, KS-WNK1 is expressed only in the kidney, where it constitutes 90% of WNK1 mRNA and is confined to the distal convoluted tubule, connecting tubule, and cortical collecting duct, suggesting that it has an important and unique function in these segments. Most of the functional studies of WNK1 were performed with the full-length L-WNK1 isoform. A recent study suggested that aldosterone upregulates KS-WNK1 in collecting duct cells, leading to ENaC activation (9), but this is at odds with studies from the Cobb group indicating that it is the NH2-terminal region unique to L-WNK1 that activates ENaC, via regulation of SGK1 (22, 23). Thus the role of KS-WNK1 remains a mystery.

In this issue of the American Journal of Physiology-Renal Physiology, Subramanya and colleagues (14) have solved an important piece of the puzzle. They show that KS-WNK1 has no direct effect on WNK4 or NCC but, instead, binds to, and acts as a dominant-negative regulator of, L-WNK1. This makes intuitive sense because KS-WNK1 lacks the kinase domain but retains downstream portions of the WNK1 protein that are postulated to participate in protein-protein interactions. KS-WNK1 inhibits the kinase activity of L-WNK1, and it abrogates the ability of L-WNK1 to inhibit WNK4-mediated downregulation of NCC. Thus KS-WNK1, via this complex inhibitory cascade, ultimately reduces NaCl reabsorption.

As with any good study, this one raises as many questions as it answers. First, why does WNK1 in the distal nephron need this extra layer of regulation whereas all other tissues in the body do not? Presumably it must be related to the need to tightly regulate NaCl reabsorption in this part of the renal tubule. The fact that KS-WNK1 expression coincides with the nephron segments that are mineralocorticoid responsive makes the hypothesis of Naray-Fejes-Toth et al. (9) that KS-WNK1 is an aldosterone-regulated gene quite attractive. However, the prediction that aldosterone upregulation of KS-WNK1 would reduce surface NCC abundance contradicts observations that aldosterone actually upregulates NCC (4, 16). Second, could KS-WNK1 be regulated in other ways, for example, by blood pressure or sodium balance, and be an important signal in physiological modulation of NCC function? Third, what is the effect of deletion of the first intron in PHAII patients on the activity of the promoter in the intron 4 that directs expression of KS-WNK1? Perhaps alterations in KS-WNK1 expression play a role in the phenotype of this disease. Fourth, KS-WNK1 is not just missing a kinase domain. It also has a unique short NH2 terminal encoded by exon 4a that is unusually rich in cysteine residues (1, 10). Could this play some role in KS-WNK1 function? Finally, a number of other lower abundance splice variants of WNK1 have been described, including one with a slightly shorter NH2 terminal due to alternative promoter use at the first exon, and alternative splice variants at exons 9, 11, and 12 (1, 10). Could these also have important functional roles? We look forward to future studies that will further unravel the complexities underlying how the WNK proteins regulate renal tubule salt transport.
REFERENCES


