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WNK kinases and the kidney

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Abstract

In the kidney, the renal tubule plays a major role in maintaining fluid and electrolyte balance. This balance is achieved by an interplay between various hormones and nerves that signal changes throughout the body and transfer these signals to transport proteins. Increased or reduced activity of these transporters helps to restore homeostasis, but can also contribute to disease (e.g. sodium retention in hypertension). In recent years, it has become clear that the signal transfer to transporters is largely mediated by kinases. Among these, WNK kinases (With No lysine = K) stand out, because they regulate the major sodium and potassium transporters in the distal nephron. Moreover, mutations in genes encoding WNK kinases result in an inherited form of salt-sensitive hypertension with hyperkalemia, illustrating their important role in sodium, potassium, and blood pressure regulation. More recently, WNK kinases were found to play a role in acquired forms of hypertension as well. Together, the evolving insight in the kinase regulation of ion transport is providing new insights in the longstanding question how salt and blood pressure are related. Here, we review the current models of how WNK kinases regulate the various transport proteins and which roles they play in health and disease.

Keywords

Distal tubule; WNK kinases; Hypertension; NaCl transport; SPAK; Pseudohypoaldosteronism type 2

Introduction

WNK kinases (WNKs) are atypical protein kinases with pleiotropic effects [1]. WNKs are atypical, because the catalytic lysine crucial for binding to ATP is located in subdomain I instead of II, where it is located in all other serine/threonine kinases. WNKs owe their name to this unusual placement: With No K, where K stands for the amino acid lysine. WNKs have pleiotropic effects, because they are expressed in several tissues where they mediate processes as diverse as solute transport, neurotransmission, and cell growth. Four genes encode the WNK kinases in humans, *WNK1*, *WNK2*, *WNK3*, and *WNK4*. This review will focus on the role of WNKs in the kidney, primarily their effects on ion transport along the distal nephron. WNKs regulate three sodium transporters, the sodium potassium chloride cotransporter type 2 (NKCC2), the sodium chloride cotransporter (NCC), and the epithelial sodium channel (ENaC). Furthermore, WNKs regulate the aldosterone-regulated renal outer

medullary potassium channel (ROMK). Because the distal nephron is sensitive to several homeo-static hormones, WNKs can be regarded as molecular switches that modify transporter activity depending on physiological demands. As such, they play important roles in regulating sodium, potassium, and blood pressure. Besides these physiological roles, mutations in WNKs cause familial hyperkalemic hypertension (OMIM 145260), a rare monogenetic disease that is also known as pseudohypoaldosteronism type 2 or Gordon syndrome [2]. The association of WNKs with human disease has sparked clinical interest and subsequent studies have revealed that WNKs may play roles in acquired forms of hypertension. On the other hand, it has also become increasingly clear that WNKs are not the only kinases regulating ion transport. In fact, WNKs appear to be part of a larger kinase network, including other kinases such as the Ste20-related kinase SPAK and serum and glucocorticoid inducible kinase 1 (SGK1). In this review, we will first review the current models of regulation of NKCC2, ROMK, NCC, and ENaC and by WNKs. This is followed by an integrated discussion on the role of WNKs in health and disease.

Regulation of ion transport by WNKs

Regulation of the NKCC2

The sodium potassium chloride cotransporter type 2 (NKCC2) is the main sodium transporter in the thick ascending limb (TAL) of the loop of Henle (Fig. 1). In this part of the renal tubule, active sodium chloride reabsorption takes place to generate a concentration gradient for subsequent water reabsorption in the collecting duct. Potassium is also transported by the NKCC2, but the majority of potassium recycles to the tubular lumen, via ROMK. This recycling step is important because it generates a positive epithelial voltage that stimulates paracellular transport of sodium, calcium, and magnesium, via tight junction proteins called claudins. Inactivating mutations of NKCC2 result in a relatively severe phenotype called Bartter syndrome, a salt-wasting disorder that is also characterized by hypokalemic alkalosis. With regard to WNK-regulation of NKCC2, some data point towards a role for WNK3 in regulating NKCC2. Rinehart and co-workers identified WNK3 as a positive regulator of NKCC2 trafficking and phosphorylation [3] (Fig. 2A). Ion transport by the TAL is under the control of vasopressin [4], and WNK3 enhanced the vasopressin-stimulated phosphorylation of NKCC2 at threonines 184 and 189 [3]. Ponce-Coria and colleagues showed that low intracellular chloride stimulated NKCC2 and that this process was also mediated by WNKs [5]. This stimulatory effect relied on an interaction between WNK3 and SPAK; kinase-dead WNK3 or elimination of WNK3's SPAK-binding motif abrogated the effect [5]. These observations may explain why mutations in the basolateral chloride transporter CIC-Kb cause Bartter syndrome; such mutations, by inhibiting chloride efflux across the basolateral membrane, will increase intracellular chloride and inhibit NKCC2 [5]. Recently, it was shown that the predominant SPAK form along the TAL is kinase deficient (called KS-SPAK) and acts to inhibit OSR1, which stimulates NKCC2 [6].

Regulation of the ROMK

The renal outer medullary potassium channel (ROMK) is expressed along the entire distal nephron (Fig. 1). In the collecting duct, aldosterone activates ROMK through SGK1 resulting in potassium secretion [7,8]. The functional significance of ROMK is illustrated by the fact that inactivating mutations in the ROMK gene also cause Bartter syndrome [9]. The first evidence for the regulation of ROMK by WNKs was found in human embryonic kidney cells, in which WNK1 inhibited ROMK by stimulating its endocytosis [10] (Fig. 2B). WNK4 is also capable of inhibiting ROMK after being phosphorylated by SGK1 [11]. Conversely, KS-WNK1 showed an opposite response in the sense that it reversed the inhibition of ROMK by WNK1; amino-acids 1–253 of KS-WNK1 were found to be responsible for this counteracting effect [10]. Moreover, a study in oocytes demonstrated

that the acidic motif, but not the kinase domain, was required for WNK1-induced endocytosis of ROMK [12]. The acidic motif was also found to be important for the WNK4–ROMK interaction [13]. WNK1 increases, while KS-WNK1 decreases during dietary potassium restriction; this opposite response favors endocytosis of ROMK thereby preventing potassium secretion and maintaining potassium balance. Whether WNKs sense changes in dietary potassium through hormones or other signals (e.g. intracellular pH) remains to be determined. However, recently it was shown that WNK1-induced endocytosis of ROMK is mediated by insulin and insulin like growth factor 1 [14]; this process appears to rely on the phosphor-ylation of WNK1 by Akt1 and SGK1.

Regulation of the NCC

The sodium chloride cotransporter (NCC) is located in the distal convoluted tubule (DCT), which forms the first part of the aldosterone-sensitive distal nephron (Fig. 1). Besides aldosterone, angiotensin II [15], insulin [16], and vasopressin [17] also regulate NCC. Although this segment only reabsorbs 5% of the filtered amount of sodium, perturbations can have significant effects on total body sodium and blood pressure. For example, genetic diseases resulting in inactivity or over-activity of NCC are characterized by low-normal or high blood pressure [18]. Furthermore, thiazide diuretics, which directly inhibit NCC, are still among the most potent antihypertensive drugs. Regulation of NCC by WNKs was illustrated by mutations in WNK1 or WNK4 causing FHt, a disease known to be exquisitely sensitive to thiazides [2]. Indeed, a mouse model of FHt recapitulated hyperkalemic hypertension and also showed increased expression of NCC [19]. Crossbreeding this mouse with the NCC knockout mouse restored the normal phenotype, further illustrating the functional significance of NCC. Since these initial discoveries, subsequent studies have further unraveled the complex interactions between the various WNKs, their interactions with other kinases and their concerted action in regulating NCC (Fig. 2C). WNKs modulate both the “trafficking” and phosphorylation of NCC [18]. The regulation of NCC trafficking by WNKs involves a sequential inhibitory cascade, in which KSWNK1 inhibits WNK1, WNK1 inhibits WNK4 [20], and WNK4 inhibits NCC [21]. The inhibition of NCC by WNK4 is not caused by endocytosis [22], but rather by promoting lysosomal degradation [23]. WNK3 also stimulates NCC, just as it does NKCC2 [3]. WNK3 and WNK4 not only have divergent effects on NCC, they also antagonize each other [24]; it therefore appears to be the ratio between WNK3 and WNK4 that determines the net effect on NCC. The phosphorylation of NCC is mediated by SPAK [25]; several WNKs interact with SPAK and therefore indirectly control the phosphorylation-step of NCC. Interactions between SPAK and WNK1 [25], WNK3 [26], and WNK4 [27] have been reported. Interestingly, a recent study showed that a brain and kidney isoform of WNK3 exists; only the latter can activate NCC through a SPAK-independent mechanism [26].

Regulation of the ENaC

The epithelial sodium channel (ENaC) is the main sodium transporter in the connecting tubule and the collecting duct (Fig. 1) and comprises α -, β -, and γ -subunits. It is also expressed along the late segment of the DCT (DCT2) with NCC. ENaC is regulated by a signaling complex that consists of the ubiquitin ligase Nedd4-2, Raf-1, SGK1, and Raf-1-interacting protein glucocorticoid-induced leucine zipper (GILZ1) [28]. ENaC expression is reduced by Nedd4-2 and Raf-1, which themselves are inhibited by the aldosterone-inducible proteins GILZ1 and SGK1. Loss-of-function mutations in the α - or β -subunits of ENaC result in pseudohypoaldosteronism type 1, a severe neonatal disease of salt wasting and hyperkalemia. Conversely, gain-of-function mutations in ENaC can affect interactions with Nedd4-2, resulting in a failure to degrade the channel; the corresponding disease is called Liddle syndrome and is characterized by hypertension and hypokalemia. WNKs have been shown to regulate ENaC primarily by interacting with SGK1. For example, WNK1 activates

SGK1 via a noncatalytic mechanism [29], and a recent study suggested that all four WNKs can interact with SGK1 [30]. Despite these insights, the physiological role of WNK kinases in regulating ENaC remains opaque [30].

Regulation of other transporters by WNKs

Several other kidney transport proteins are regulated by WNKs, including the transient receptor potential vanilloid channels TRPV4, TRPV5, TRPV6 [31–33], and the potassium channel Maxi-K (also called BK or slo1) [34]. TRPV4 is a nonselective cation channel located in the distal nephron; its precise function has not been completely clarified. TRPV4 is functionally, but not physically, regulated by WNK1 and WNK4 [32]. TRPV5 is the gatekeeper of transcellular calcium reabsorption and is located in the DCT and CNT. WNK4 was not only shown to enhance the endocytosis of TRPV5, but also to increase the response to protein kinase C (PKC), which is stimulated by parathyroid hormone [31]. Conversely, WNK3 stimulates TRPV5 as well as TRPV6 via a kinase-dependent mechanism [33]. Collectively, these data indicate that WNKs regulate renal calcium handling, although it remains unclear if this also provides an explanation for hypercalciuria in familial hyperkalemic hypertension (see further) [35].

WNKs in health and disease

The aldosterone paradox

The WNKs have been characterized as ‘molecular switches’ based on their capacity to regulate sodium and potassium transporters differentially. Hypovolemia and hyperkalemia elicit different constellations of responses along the distal nephron to maintain homeostasis. During hypovolemia, the extracellular fluid volume needs to be protected to guarantee blood pressure and organ perfusion. Hypovolemia activates the renin angiotensin system, enhancing aldosterone secretion; aldosterone promotes sodium reabsorption. Conversely, during hyperkalemia, potassium secretion is stimulated to avoid cardiac and neuromuscular complications; this process is also mediated by aldosterone. The observation that a single hormone, aldosterone, has different effects on renal sodium and potassium transport, depending on the physiological situation, has been termed the “aldosterone paradox”. To explain the aldosterone paradox, it is necessary to consider the factors accompanying hypovolemia and hyperkalemia. Although hypovolemia and hyperkalemia are both characterized by elevated aldosterone, only hypovolemia is associated with a concomitant rise in angiotensin II. Angiotensin II has been shown to abrogate the inhibitory effects of WNK4 [36], permitting NCC to traffic to the plasma membrane [37], and be phosphorylated by SPAK, enhancing electroneutral NaCl transport. Increased sodium reabsorption in the DCT will reduce the delivery of sodium to the collecting duct, limiting sodium-coupled potassium secretion in that segment. Recently, it was also shown that angiotensin II inhibits ROMK [38], an effect mediated both by ROMK phosphorylation and by Src-mediated phosphorylation of WNK4. The phosphorylation of WNK4 abrogates the stimulatory effect of non-phosphorylated WNK4 on ROMK [38,39]. Together, these effects favor electroneutral sodium reabsorption while preventing potassium secretion. Hyperkalemia, on the other hand, stimulates K secretion directly [40]. In addition, high dietary potassium increases KS-WNK1 and WNK4 [40]; this will inhibit NCC directly [41] and activates ROMK [42], therefore favoring Na reabsorption in exchange for potassium. Thus, hypovolemia and hyperkalemia are able to influence WNKs in such a way that they either stimulate sodium chloride retention or potassium secretion; this represents a unique system to maintain the electrolyte equilibrium.

Familial hyperkalemic hypertension

The initial interest in WNKs was fueled by finding that mutations in WNKs could directly cause hypertension [2]. Familial hyperkalemic hypertension (FHHt) is a rare autosomal dominant disorder that can either be caused by intronic deletions affecting WNK1 expression patterns, or by missense mutations causing dysfunctional WNK4 [43]. According to the current model, overexpression of WNK1 will activate both NCC and ENaC, thereby leading to increased sodium reabsorption at two sites. Mutant WNK4 has also been shown to stimulate ENaC [11]. How mutant WNK4 stimulates NCC, however, is still somewhat unresolved. Four models have been postulated [1], including (a) mutant WNK4 leading to loss of inhibition of NCC by wild-type WNK4, (b) mutant WNK4 exerting a dominant-negative effect on wild-type WNK4's inhibition of WNK3, (c) wild-type but not mutant WNK4 requiring angiotensin II to convert from an inhibitory to a stimulatory mode, (d) wild-type WNK4 already being stimulatory for NCC with mutant WNK4 having a gain of function. Future studies will be needed to confirm which model turns out to be most valid; these models also illustrate that the regulation of NCC by WNK4 in general remains incompletely understood. The reported effects of WNKs on ROMK help explain why FHHt is accompanied by hyperkalemia. That is, wild-type WNK1 enhances the endocytosis of ROMK; therefore, overexpression of WNK1 likely augments this effect. Surprisingly, mice expressing mutant WNK4 show normal ROMK labeling [19], thus, the molecular basis for the interaction between mutant WNK4 and ROMK remains poorly understood. Besides hyperkalemia and hypertension, patients with FHHt also exhibit renal tubular acidosis and hypercalciuria. The exact pathogenesis of these features is less clear. For example, it is unknown whether WNKs also regulate proton pumps in the collecting duct [44]. Although WNK4 stimulates the endocytosis of TRPV5 [31], a mouse model of FHHt did not show reduced TRPV5 nor TRPV6 expression [35]. Instead, TRPV6 and calbindin were *increased*. Because the investigators observed reduced NKCC2, they postulated that this was responsible for hypercalciuria, and interpreted increased TRPV6 and calbindin as secondary effects. Besides this monogenetic form of hypertension, polymorphisms in WNK genes have also been associated with blood pressure and urine potassium variation in the general population [45].

Acquired forms of hypertension

WNKs have also been associated with acquired forms of hyper-tension, including hypertension associated with potassium depletion and hyperinsulinemia (for review, see [18]). More recently, WNKs were found to play a role in two other forms of acquired hypertension, hypertension resulting from calcineurin inhibitors (CNIs) [46] and owing to sympathetic nerve activity [47]. CNIs are commonly used drugs for the prevention of rejection of transplanted organs, or for the treatment of autoimmune disease. Their use is complicated by hypertension and a range of renal tubular disorders, including hyperkalemia, renal tubular acidosis, and hypercalciuria. In fact, these characteristics bear a striking resemblance with FHHt, raising the possibility that WNKs could play a role. We recently addressed this hypothesis in animals and patients [46]. We first demonstrated that mice treated with the CNI tacrolimus developed salt-sensitive hyper-tension, hyperkalemia, renal tubular acidosis, and hypercalciuria, recapitulating the FHHt-phenotype. We then showed that tacrolimus increased WNK3, WNK4, SPAK, and phosphorylated NCC in kidney homogenates. As more functional evidence for the involvement of NCC, we showed that tacrolimus failed to cause hypertension in NCC knockout mice, but caused an exaggerated hypertensive response in transgenic mice overexpressing NCC. Patients with CNI-induced hypertension showed a greater increase in fractional chloride excretion after receiving a thiazide diuretic, suggesting increased NCC activity. These patients also had a higher expression of NCC and phosphorylated NCC in their transplant biopsies. Together, these findings suggested CNI-induced hypertension to be, at least in part, a salt-sensitive form of

hypertension mediated by the WNK-SPAK-NCC pathway. Another group found similar results using cyclosporine in rats [48]. Therapeutically, this implies that CNI-induced hypertension could be especially sensitive to thiazide diuretics. Previously, CNI-induced hypertension was mainly considered to be caused by vasoconstriction. However, salt retention and vasoconstriction may not be independent mechanisms for CNI-induced hypertension, as patients with salt wasting syndromes, such as Gitelman syndrome (caused by inactivating mutations of NCC), exhibit reduced vascular contractility, even though NCC is not expressed in vascular tissue [49]. How renal sodium retention is transferred to altered vascular reactivity remains to be elucidated. Of interest, in this regard, is the demonstration that the kinase SPAK is not only expressed in kidney, but also in vascular tissue, where it regulates contractility through an effect on NKCC1 [50]. These studies suggest a common pathway for mechanisms of hypertension that were considered to be distinct. Another recent study illustrated something similar by showing that sodium retention induced by sympathetic nerve activity is also mediated by WNKs [47]. In this study, WNK4 gene transcription was reduced through an interaction between the β_2 -adrenergic receptor and the glucocorticoid receptor, thereby increasing NCC activity. This mechanism represents a remarkable interaction between nervous, endocrine, and renal systems. Thus, these examples illustrate that since the initial discovery of mutations in WNKs causing FHHt, increasingly more roles for these kinases in the mechanism of disease are being identified.

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REFERENCES

1. McCormick JA, Ellison DH. The WNKs: atypical protein kinases with pleiotropic actions. *Physiol. Rev.* 2011; 91:177–219. [PubMed: 21248166]
2. Wilson, FH.; Disse-Nicodeme, S.; Choate, KA.; Ishikawa, K.; Nelson-Williams, C.; Desitter, I.; Gunel, M.; Milford, DV.; Lipkin, GW.; Achard, JM.; Feely, MP.; Dussol, B.; Berland, Y.; Unwin, RJ.; Mayan, H.; Simon, DB.; Farfel, Z.; Jeunemaitre, X.; Lifton, RP. *Science*. Vol. 293. New York, N.Y.: 2001. Human hypertension caused by mutations in WNK kinases; p. 1107-1112.
3. Rinehart J, Kahle KT, de Los Heros P, Vazquez N, Meade P, Wilson FH, Hebert SC, Gimenez I, Gamba G, Lifton RP. WNK3 kinase is a positive regulator of NKCC2 and NCC, renal cation-Cl-cotransporters required for normal blood pressure homeostasis. *Proc. Natl. Acad. Sci. U. S. A.* 2005; 102:16777–16782. [PubMed: 16275913]
4. Gimenez I, Forbush B. Short-term stimulation of the renal Na–K–Cl cotransporter (NKCC2) by vasopressin involves phosphorylation and membrane translocation of the protein. *J. Biol. Chem.* 2003; 278:26946–26951. [PubMed: 12732642]
5. Ponce-Coria J, San-Cristobal P, Kahle KT, Vazquez N, Pacheco-Alvarez D, de Los Heros P, Juarez P, Munoz E, Michel G, Bobadilla NA, Gimenez I, Lifton RP, Hebert SC, Gamba G. Regulation of NKCC2 by a chloride-sensing mechanism involving the WNK3 and SPAK kinases. *Proc. Natl. Acad. Sci. U. S. A.* 2008; 105:8458–8463. [PubMed: 18550832]
6. McCormick JA, Mutig K, Nelson JH, Saritas T, Hoorn EJ, Yang CL, Rogers S, Curry J, Delpire E, Bachmann S, Ellison DH. A SPAK isoform switch modulates renal salt transport and blood pressure. *Cell Metab.* 2011; 14(3):352–364. [PubMed: 21907141]
7. Wald H, Garty H, Palmer LG, Popovtzer MM. Differential regulation of ROMK expression in kidney cortex and medulla by aldosterone and potassium. *Am. J. Physiol.* 1998; 275:F239–F245. [PubMed: 9691014]
8. Yoo D, Kim BY, Campo C, Nance L, King A, Maouyo D, Welling PA. Cell surface expression of the ROMK (Kir 1.1) channel is regulated by the aldosterone-induced kinase, SGK-1, and protein kinase A. *J. Biol. Chem.* 2003; 278:23066–23075. [PubMed: 12684516]

9. Simon DB, Karet FE, Rodriguez-Soriano J, Hamdan JH, DiPietro A, Trachtman H, Sanjad SA, Lifton RP. Genetic heterogeneity of Bartter's syndrome revealed by mutations in the K⁺ channel. ROMK, Nat. Genet. 1996; 14:152–156.
10. Lazrak A, Liu Z, Huang CL. Antagonistic regulation of ROMK by long and kidney-specific WNK1 isoforms. Proc. Natl. Acad. Sci. U. S. A. 2006; 103:1615–1620. [PubMed: 16428287]
11. Ring AM, Cheng SX, Leng Q, Kahle KT, Rinehart J, Lalioti MD, Volkman HM, Wilson FH, Hebert SC, Lifton RP. WNK4 regulates activity of the epithelial Na⁺ channel in vitro and in vivo. Proc. Natl. Acad. Sci. U. S. A. 2007; 104:4020–4024. [PubMed: 17360470]
12. Cope G, Murthy M, Golbang AP, Hamad A, Liu CH, Cuthbert AW, O'Shaughnessy KM. WNK1 affects surface expression of the ROMK potassium channel independent of WNK4. J. Am. Soc. Nephrol. 2006; 17:1867–1874. [PubMed: 16775035]
13. Murthy M, Cope G, O'Shaughnessy KM. The acidic motif of WNK4 is crucial for its interaction with the K channel ROMK. Biochem. Biophys. Res. Commun. 2008; 375:651–654. [PubMed: 18755144]
14. Cheng CJ, Huang CL. Activation of PI3-kinase stimulates endocytosis of ROMK via Akt1/SGK1-dependent phosphorylation of WNK1. J. Am. Soc. Nephrol. 2011; 22:460–471. [PubMed: 21355052]
15. van der Lubbe N, Lim CH, Fenton RA, Meima ME, Jan Danser AH, Zietse R, Hoorn EJ. Angiotensin II induces phosphorylation of the thiazide-sensitive sodium chloride cotransporter independent of aldosterone. Kidney Int. 2011; 79:66–76. [PubMed: 20720527]
16. Song J, Hu X, Riaz S, Tiwari S, Wade JB, Ecelbarger CA. Regulation of blood pressure, the epithelial sodium channel (ENaC), and other key renal sodium transporters by chronic insulin infusion in rats. Am. J. Physiol. 2006; 290:F1055–F1064.
17. Pedersen NB, Hofmeister MV, Rosenbaek LL, Nielsen J, Fenton RA. Vasopressin induces phosphorylation of the thiazide-sensitive sodium chloride cotransporter in the distal convoluted tubule. Kidney Int. 2010; 78:160–169. [PubMed: 20445498]
18. Hoorn EJ, Nelson JH, McCormick JA, Ellison DH. The WNK kinase network regulating sodium, potassium, and blood pressure. J. Am. Soc. Nephrol. 2011; 22:605–614. [PubMed: 21436285]
19. Lalioti MD, Zhang J, Volkman HM, Kahle KT, Hoffmann KE, Toka HR, Nelson-Williams C, Ellison DH, Flavell R, Booth CJ, Lu Y, Geller DS, Lifton RP. Wnk4 controls blood pressure and potassium homeostasis via regulation of mass and activity of the distal convoluted tubule. Nat. Genet. 2006; 38:1124–1132. [PubMed: 16964266]
20. Yang CL, Zhu X, Wang Z, Subramanya AR, Ellison DH. Mechanisms of WNK1 and WNK4 interaction in the regulation of thiazide-sensitive NaCl cotransport. J. Clin. Investig. 2005; 115:1379–1387. [PubMed: 15841204]
21. Yang CL, Angell J, Mitchell R, Ellison DH. WNK kinases regulate thiazide-sensitive Na–Cl cotransport. J. Clin. Investig. 2003; 111:1039–1045. [PubMed: 12671053]
22. Golbang AP, Cope G, Hamad A, Murthy M, Liu CH, Cuthbert AW, O'Shaughnessy KM. Regulation of the expression of the Na/Cl cotransporter by WNK4 and WNK1: evidence that accelerated dynamin-dependent endocytosis is not involved. Am. J. Physiol. 2006; 291:F1369–F1376.
23. Subramanya AR, Liu J, Ellison DH, Wade JB, Welling PA. WNK4 diverts the thiazide-sensitive NaCl cotransporter to the lysosome and stimulates AP-3 interaction. J. Biol. Chem. 2009; 284:18471–18480. [PubMed: 19401467]
24. Yang CL, Zhu X, Ellison DH. The thiazide-sensitive Na–Cl cotransporter is regulated by a WNK kinase signaling complex. J. Clin. Investig. 2007; 117:3403–3411. [PubMed: 17975670]
25. Moriguchi T, Urushiyama S, Hisamoto N, Iemura S, Uchida S, Natsume T, Matsumoto K, Shibuya H. WNK1 regulates phosphorylation of cation–chloride-coupled cotransporters via the STE20-related kinases, SPAK and OSR1. J. Biol. Chem. 2005; 280:42685–42693. [PubMed: 16263722]
26. Glover M, Zuber AM, O'Shaughnessy KM. Renal and brain isoforms of WNK3 have opposite effects on NCCT expression. J. Am. Soc. Nephrol. 2009; 20:1314–1322. [PubMed: 19470686]
27. San-Cristobal P, Ponce-Coria J, Vazquez N, Bobadilla NA, Gamba G. WNK3 and WNK4 amino-terminal domain defines their effect on the renal Na⁺–Cl-cotransporter. Am. J. Physiol. 2008; 295:F1199–F1206.

28. Soundararajan R, Melters D, Shih IC, Wang J, Pearce D. Epithelial sodium channel regulated by differential composition of a signaling complex. *Proc. Natl. Acad. Sci. U. S. A.* 2009; 106:7804–7809. [PubMed: 19380724]
29. Xu BE, Stippec S, Chu PY, Lazrak A, Li XJ, Lee BH, English JM, Ortega B, Huang CL, Cobb MH. WNK1 activates SGK1 to regulate the epithelial sodium channel. *Proc. Natl. Acad. Sci. U. S. A.* 2005; 102:10315–10320. [PubMed: 16006511]
30. Heise CJ, Xu BE, Deaton SL, Cha SK, Cheng CJ, Earnest S, Sengupta S, Juang YC, Stippec S, Xu Y, Zhao Y, Huang CL, Cobb MH. Serum and glucocorticoid-induced kinase (SGK) 1 and the epithelial sodium channel are regulated by multiple with no lysine (WNK) family members. *J. Biol. Chem.* 2010; 285:25161–25167. [PubMed: 20525693]
31. Cha SK, Huang CL. WNK4 kinase stimulates caveola-mediated endocytosis of TRPV5 amplifying the dynamic range of regulation of the channel by protein kinase C. *J Biol Chem.* 2010; 285:6604–6611. [PubMed: 20061383]
32. Fu Y, Subramanya A, Rozansky D, Cohen DM. WNK kinases influence TRPV4 channel function and localization. *Am. J. Physiol.* 2006; 290:F1305–F1314.
33. Zhang W, Na T, Peng JB. WNK3 positively regulates epithelial calcium channels TRPV5 and TRPV6 via a kinase-dependent pathway. *Am. J. Physiol.* 2008; 295:F1472–F1484.
34. Zhuang J, Zhang X, Wang D, Li J, Zhou B, Shi Z, Gu D, Denson DD, Eaton DC, Cai H. Wnk4 kinase inhibits Maxi K channel activity by a kinase-dependent mechanism. *Am. J. Physiol.* 2011; 301:F410–F419.
35. Yang SS, Hsu YJ, Chiga M, Rai T, Sasaki S, Uchida S, Lin SH. Mechanisms for hypercalciuria in pseudohypoaldosteronism type II-causing WNK4 knock-in mice. *Endocrinology.* 2010; 151:1829–1836. [PubMed: 20181799]
36. San-Cristobal P, Pacheco-Alvarez D, Richardson C, Ring AM, Vazquez N, Rafiqi FH, Chari D, Kahle KT, Leng Q, Bobadilla NA, Hebert SC, Alessi DR, Lifton RP, Gamba G. Angiotensin II signaling increases activity of the renal Na–Cl cotransporter through a WNK4-SPAK-dependent pathway. *Proc. Natl. Acad. Sci. U. S. A.* 2009; 106:4384–4389. [PubMed: 19240212]
37. Sandberg MB, Riquier AD, Pihakaski-Maunsbach K, McDonough AA, Maunsbach AB. ANG II provokes acute trafficking of distal tubule Na⁺–Cl cotransporter to apical membrane. *Am. J. Physiol. Ren. Physiol.* 2007; 293:F662–F669.
38. Yue P, Sun P, Lin DH, Pan C, Xing W, Wang W. Angiotensin II diminishes the effect of SGK1 on the WNK4-mediated inhibition of ROMK1 channels. *Kidney Int.* 2011; 79:423–431. [PubMed: 20927043]
39. Yue P, Lin DH, Pan CY, Leng Q, Giebisch G, Lifton RP, Wang WH. Src family protein tyrosine kinase (PTK) modulates the effect of SGK1 and WNK4 on ROMK channels. *Proc. Natl. Acad. Sci. U. S. A.* 2009; 106:15061–15066. [PubMed: 19706464]
40. O'Reilly M, Marshall E, Macgillivray T, Mittal M, Xue W, Kenyon CJ, Brown RW. Dietary electrolyte-driven responses in the renal WNK kinase pathway in vivo. *J. Am. Soc. Nephrol.* 2006; 17:2402–2413. [PubMed: 16899520]
41. Subramanya AR, Yang CL, Zhu X, Ellison DH. Dominant-negative regulation of WNK1 by its kidney-specific kinase-defective isoform. *Am. J. Physiol. Ren. Physiol.* 2006; 290:F619–F624.
42. Wade JB, Fang L, Liu J, Li D, Yang CL, Subramanya AR, Maouyo D, Mason A, Ellison DH, Welling PA. WNK1 kinase isoform switch regulates renal potassium excretion. *Proc. Natl. Acad. Sci. U. S. A.* 2006; 103:8558–8563. [PubMed: 16709664]
43. Hadchouel J, Delaloy C, Faure S, Achard JM, Jeunemaitre X. Familial hyperkalemic hypertension. *J. Am. Soc. Nephrol.* 2006; 17:208–217. [PubMed: 16221868]
44. Karet FE. Mechanisms in hyperkalemic renal tubular acidosis. *J. Am. Soc. Nephrol.* 2009; 20:251–254. [PubMed: 19193780]
45. Newhouse S, Farrall M, Wallace C, Hoti M, Burke B, Howard P, Onipinla A, Lee K, Shaw-Hawkins S, Dobson R, Brown M, Samani NJ, Dominiczak AF, Connell JM, Lathrop GM, Kooner J, Chambers J, Elliott P, Clarke R, Collins R, Laan M, Org E, Juhanson P, Veldre G, Viigimaa M, Eyheramendy S, Cappuccio FP, Ji C, Iacone R, Strazzullo P, Kumari M, Marmot M, Brunner E, Caulfield M, Munroe PB. Polymorphisms in the WNK1 gene are associated with blood pressure variation and urinary potassium excretion. *PLoS One.* 2009; 4:e5003. [PubMed: 19347040]

46. Hoorn EJ, Walsh SB, McCormick JA, Fürstenberg A, Yang CL, Roeschel T, Paliege A, Howie AJ, Conley J, Bachmann S, Unwin RJ, Ellison DH. The calcineurin inhibitor tacrolimus activates the renal sodium chloride cotransporter to cause hypertension. *Nat. Med.* Oct 2; 2011 17(10):1304–1309. [PubMed: 21963515]
47. Mu S, Shimosawa T, Ogura S, Wang H, Uetake Y, Kawakami-Mori F, Marumo T, Yatomi Y, Geller DS, Tanaka H, Fujita T. Epigenetic modulation of the renal beta-adrenergic-WNK4 pathway in salt-sensitive hypertension. *Nat. Med.* 2011; 17:573–580. [PubMed: 21499270]
48. Melnikov S, Mayan H, Uchida S, Holtzman EJ, Farfel Z. Cyclosporine metabolic side effects: association with the WNK4 system. *Eur. J. Clin. Invest.* 2011; 41:1113–1120. [PubMed: 21434893]
49. Ellison DH, Loffing J. Thiazide effects and adverse effects: insights from molecular genetics. *Hypertension.* 2009; 54:196–202. [PubMed: 19564550]
50. Yang SS, Lo YF, Wu CC, Lin SW, Yeh CJ, Chu P, Sytwu HK, Uchida S, Sasaki S, Lin SH. SPAK-knockout mice manifest Gitelman syndrome and impaired vasoconstriction. *J. Am. Soc. Nephrol.* 2010; 21:1868–1877. [PubMed: 20813865]

Fig. 1.

Distribution of transport proteins (blue, abbreviations defined in the text), 11 β -OH steroid dehydrogenase type 2 (11 HSD2 in pink), WNK kinases (light green, abbreviations in text), and SPAK/OSR1, including KS-SPAK (red) along the nephron. The segments are the thick ascending limb (TAL), distal convoluted tubule, with early and late segments (DCT1 and DCT2), and connecting tubule/collecting duct (CNT/CD).

Fig. 2. Simplified models of WNK effects on ion transport proteins along thick ascending limb (TAL), distal convoluted tubule (DCT) and connecting tubule/collecting duct (CNT/CD). Along TAL, WNK (? WNK3) may stimulate trafficking to the membrane and phosphorylation via OSR1. This process is inhibited by KS-SPAK. Along the DCT, NCC movement of the plasma membrane is inhibited by WNK4, which its phosphorylation is mediated by SPAK, via WNK kinases. Along the CNT/CD, WNK4 and WNK1 both accelerate endocytosis of ROMK. Many effects on ENaC have been described, but these remain poorly characterized.