WNK Kinases and Renal Sodium Transport in Health and Disease
An Integrated View

James A. McCormick, Chao-Ling Yang, David H. Ellison

Hypertension affects 25% of the adult population in the developed world and is a major independent risk factor for stroke, myocardial infarction, and heart and kidney failure. Although many genetic and environmental contributors are involved, the kidney plays a dominant role, both in animal models, and in human essential hypertension. Most monogenic hypertensive syndromes result from increased Na+ transport along the aldosterone-sensitive distal nephron. The majority of these, however, are associated with hypokalemia, indicating that activation of the epithelial Na+ channel, ENaC, is a primary pathophysiologic process. In contrast, familial hyperkalemic hypertension (FHHt; also known as Gordon’s syndrome or type II pseudohypoaldosteronism) is characterized by hypertension with hyperkalemia, indicating that stimulated ENaC cannot be the primary event. FHHt was first described in 1964 and later shown to be inherited in an autosomal dominant manner. Patients with FHHt exhibit hyperkalemia, which seems to be the most consistent feature of the disease. Hypertension, although commonly present and sometimes severe, often appears later in the natural history. Other characteristic features include mild metabolic acidosis, suppressed plasma renin activity, and aldosterone levels that are lower than would be expected, considering the hyperkalemia. Infusing the chloride salt of NaCl/H11001 does not increase urinary potassium excretion in patients with FHHt, as it does in the normal individual, whereas infusing nonchloride salts of Na+ do increase K+ excretion in FHHt patients to normal levels. Patients are often remarkably sensitive to thiazide diuretics, which can correct both the hyperkalemia and hypertension, in many cases.

WNK Kinases and FHHt

In 2001, some cases of FHHt were shown to result from mutations in WNK1 and WNK4, identifying WNK kinases as previously undiscovered components of a novel electrolyte homeostasis pathway. Since that time, information about the physiological role of WNK kinases, their substrates and mechanisms of action, and their role in FHHt has accumulated rapidly. The identification of a previously unrecognized signal transduction pathway that plays a central role in renal electrolyte balance and blood pressure control has generated considerable interest and has been reviewed widely. Despite this, several fundamental questions regarding physiological and pathophysiological actions of WNK kinases and the pathogenesis of FHHt are only now being addressed. The focus of this review is several of these remaining questions.

WNK1 was first identified by Xu et al during a search for novel members of the mitogen-activated protein/extracellular signal–regulated protein kinase family. They named the new family of kinases “with no lysine” (K), because the lysine critical for ATP binding in the catalytic site is in subdomain 1 rather than subdomain 2, where it is located in other serine/threonine kinases. When first identified, the function, upstream regulators, and downstream targets of the WNK kinases were not clear. Xu et al demonstrated that WNKs are catalytically active and widely expressed, at least at the transcript level. Four WNKs have now been identified in humans (WNK1 through WNK4), and all exhibit a similar domain structure (see Figure 1), including a short amino-terminal domain, a highly conserved kinase domain, and a longer carboxyl-terminal domain, with ≥2 coiled coil domains. Like many other kinases, WNKs contain an autoinhibitory domain that inhibits kinase activity; this domain, which contains 2 crucial phenylalanine residues, lies just beyond the kinase domain (Figure 1). In addition, there are several PXPF motifs that may interact with SH3 domains of other proteins. Two serine residues located within the activation loop (S382 and S378) modulate kinase activity.

WNK kinases seem to be involved in many physiological processes (Table). WNK1 is activated by both hypertonicity and hypotonicity and is sensitive to osmotic stressors, such as NaCl, KCl, and carbohydrates. This suggests that WNK kinases participate in cell volume regulation, because they also interact with, phosphorylate, and activate the tonicity-responsive kinases Ste-20 related proline-alanine-rich kinase (SPAK/PASK) and oxidative stress-response kinase 1 (OSR1), thereby affecting cation chloride cotransporters. WNK kinases also bind to and phosphorylate synaptotagmin-

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2,23 a protein that regulates membrane fusion events and participates in neurotransmission. WNK kinases affect cell growth and apoptosis through several pathways.24–26 WNK1 is expressed ubiquitously, and its expression in the cardiovascular system in particular may account for the lethality of WNK1 deletion in utero.28 WNK3 is highly expressed in the brain and may play an important role in the regulation of volume and intracellular Cl\(^{-}\) in γ-aminobutyric acid–producing neurons.29,29 Finally, WNK kinases modulate ion transport across epithelia (see below).

Despite these diverse and essential functions, WNK kinase mutations identified in humans cause a phenotype that results predominantly from kidney dysfunction, although minor effects in other organ systems may be present.30 Disease-causing WNK1 mutations are large deletions in the first intron that do not change the coding sequence.30 These intronic mutations are believed to increase WNK1 protein expression,30 although this observation has not been corroborated in renal tissue. FHHt-causing WNK4 mutations are missense mutations located within 2 discrete regions (see Figure 1), the first within an acidic motif adjacent to the first coiled coil domain and the second adjacent to the second coiled coil domain.10,31 The FHHt-causing WNK4 mutations lie outside the kinase domain.

Expression and Regulation of WNK Kinases in the Kidney

To understand how the WNK kinases regulate electrolyte homeostasis and how this relates to FHH and normal physiology, it is important to understand the expression pattern of ion transport proteins in the distal nephron. The distal tubule (see Figure 2) can be defined as the region of the nephron between the macula densa and the confluence with another tubule to form the collecting duct. This region is composed of a short segment of thick ascending limb, the true distal convoluted tubule (DCT), the connecting tubule (CNT), and the initial segment of cortical collecting tubule (for a review, see Reference 32). The thiazide-sensitive Na\(^{-}\)/Cl\(^{-}\} cotransporter (NCC) has been localized exclusively to the DCT at the mRNA level using in situ hybridization33–35 and single-nephron PCR.36 At the protein level, NCC expression is also limited to DCT cells37,38; NCC expression, therefore, “defines” the DCT. Further expression analysis has revealed that the DCT can be subdivided into an “early” DCT (DCT1) and a “late” DCT (DCT2).34 Both DCT1 and DCT2 express the NCC, but the DCT1 does not express the sodium-calcium exchanger (Na/Ca)34 or ENaC,39 both of which are expressed along the DCT2.40 In contrast, the collecting duct, while expressing ENaC, does not express Na/Ca.34 The K channel, ROMK, and the Na-K-ATPase are expressed all along the distal tubule.41

The WNK1 gene (WNK1 or PRKWNK1) produces 12 major products42–44 (and several minor products), a full-length kinase-active WNK1 (WNK1), which is widely expressed, and a second truncated product (Figure 1). The truncated product derives from a separate promoter and lacks the majority of the kinase domain; this product is, therefore, kinase inactive. It seems to be expressed only by kidney tubule epithelial cells, predominantly along the DCT and connecting tubule (see Figure 2). For this reason, the kinase-inactive isoform has been termed “kidney-specific WNK1” (KS-WNK1), to differentiate it from the full-length WNK1.

Table. Physiological Effects of WNK Kinases

<table>
<thead>
<tr>
<th>Function</th>
<th>Mediator</th>
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<tr>
<td>Cell volume regulation</td>
<td>OSR1/SPAK11,22,35–36</td>
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<tr>
<td>Neurotransmission</td>
<td>Synaptotagmin55</td>
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<tr>
<td>Cell proliferation</td>
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<tr>
<td>Development</td>
<td>Unknown28</td>
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<tr>
<td>Paracellular permeability</td>
<td>Claudin72</td>
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<tr>
<td>Transepithelial ion transport</td>
<td>Intersectin24, clathrin53,63,72</td>
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<tr>
<td></td>
<td>SGK16,8,37 OSR1/SPAK21,22,62–84</td>
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Figure 1. Structures of WNK kinases. WNKs 1 to 4 are shown. All contain a homologous kinase domain (pink), an autoinhibitory domain (green) with 2 essential phenylalanine residues (P), and coiled coil domains (yellow). Two phosphorylated (P) serine residues that are essential for WNK1 activation are shown (S382 is more important). The alternative first exon in KS-WNK1 is shown in dark red. Regions of WNK4 mutations that cause FHHt are shown in orange. Approximate domain locations and specific residues are provided based on mouse WNKs.

Figure 2. Distribution patterns of sodium and potassium transport and aldosterone signaling machinery along the distal nephron. The figure shows the thick ascending limb (TAL), the DCT (with early and late segments), the CNT, and the cortical (C), and outer medullary (OM) collecting ducts (CD). Locations of transport proteins and regulatory kinases are shown, including the NCC, the epithelial Na\(^{+}\) channel, ENaC, the potassium channel, ROMK, WNK kinases, including kidney-specific WNK1 (KS-WNK1), full-length WNK1 (WNK1) and WNK4, serum and glucocorticoid induced kinase (SGK1), and 11-β hydroxysteroid dehydrogenase (11HSD2).
WNK4 is expressed by epithelial cells throughout the body, including cells of the distal nephron, where it localizes, at least in part, adjacent to tight junctions. It is highly expressed by cells of the DCT and connecting tubule, but expression extends distally into the collecting duct and, at lower levels, into the thick ascending limb. WNK3 is a third member of the family that is also expressed by kidney epithelial cells and elsewhere in the body. In contrast to the renal expression patterns of KS-WNK1 and WNK4, WNK3 expression is not predominant along the aldosterone-sensitive distal nephron; instead, it is expressed throughout the nephron, from the proximal tubule to the collecting duct. Although mutations in WNK3 have not been reported to be associated with FHHt, WNK3 has been shown recently to regulate the same classes of ion transport proteins that are targets of WNK1 and WNK4 (see below).

WNK Kinases Regulate NCC In Vitro

The clinical features of FHHt identify it as a disease of renal electrolyte transport, so investigation of the effects of WNK kinases was first directed at their roles in modulating renal ion transport proteins. It is now clear that WNK kinases modulate the trafficking of many transport proteins to or from the plasma membrane, at least in vitro. In view of the fact that most of the defects in FHHt can be corrected by treatment with thiazide diuretics and FHHt presents as a “mirror-image” of Gitelman’s syndrome, a disease that results from inactivating mutations of NCC, it is not surprising that WNK4 was soon shown to regulate NCC activity in vitro.

WNK4 does not affect total cellular NCC protein abundance but instead reduces NCC abundance at the plasma membrane (see Figure 3). Immunoprecipitation studies have shown that WNK4 and NCC associate in a protein complex involving the carboxyl termini of both proteins. The role of kinase activity in modulating NCC activity is controversial. Two groups reported that the effects of WNK4 on NCC are dependent on its kinase activity, whereas another group found evidence of a kinase-independent action. Indeed, our group showed that a truncated form of WNK4 lacking the entire kinase domain inhibited NCC activity. Further analysis identified a region near the carboxyl terminus of WNK4 that is required for NCC inhibition.

Studies to determine the mechanism by which WNK4 reduces surface NCC expression suggest that WNK4 inhibits the insertion of NCC into the plasma membrane, rather than affecting endocytosis. Studies in both Xenopus oocytes and mammalian cells showed that the ability of WNK4 to reduce NCC surface expression is not affected by a dominant-negative dynamin, suggesting that clathrin-dependent processes are not involved. Furthermore, the effect of WNK4 on NCC was sensitive to inhibition of lysosomal proton pumps, suggesting that WNK4 reduces trafficking of NCC to the plasma membrane, ultimately leading to enhanced lysosomal degradation.

Unlike WNK4, WNK1 does not affect NCC activity directly (see Figure 3). Instead it suppresses the effects of WNK4 on NCC. Functional studies of WNK1 have revealed that its actions to modulate WNK4 require physical association with WNK4 and intact kinase activity. The predominant renal isoform, KS-WNK1, which lacks intrinsic kinase activity, inhibits WNK1 kinase activity and inhibits its effects on NCC, presumably through a dominant-negative mechanism.

KS-WNK1 interacts physically with WNK1 which, as noted, associates with NCC.

Surprisingly, WNK3 strongly stimulates NCC activity (see Figure 3). This effect is associated with an increase in NCC protein abundance at the plasma membrane and with an increase in NCC phosphorylation. A kinase-inactive form of WNK3 exerts inhibitory effects on NCC that resemble the effects of WNK4.
Mouse Models of WNK Action

To date, 4 strains of genetically engineered mice have been generated to analyze the functions of the WNK kinases in vivo. A gene trap approach that disrupted the first WNK1 intron (and presumably left the KS-WNK1 promoter region intact) led to the production of WNK1 knockout mice.28 Mice homozygous for the disrupted allele die before embryonic day 13, possibly from cardiovascular defects. Heterozygotes were viable but had blood pressure that was significantly lower than wild-type mice, lending support to the idea that WNK1 is a stimulator of sodium reabsorption.

Lalioti et al57 generated lines of mice transgenic for wild-type WNK4 and for FHHt-causing Q562E WNK4. Animals overexpressing wild-type WNK4 had lower blood pressure than wild-type mice, whereas animals transgenic for WNK4 Q562E had higher blood pressure than wild-type mice. With regard to electrolyte balance, mice overexpressing the FHHt-causing mutant WNK4 displayed hyperkalemia to an extent similar to that seen in patients with FHHt. When challenged with a high K+ diet, Q526E WNK4 mice became more hyperkalemic, with clearly impaired K+ excretion. On a low K+ diet, wild-type WNK4 transgenic mice became hypokalemic relative to wild-type mice. Taken together, these data indicate that WNK4 plays a key role in regulating K+ balance, but the effects of WNK4 and mutant WNK4 on K+ balance are opposite.

Histological analysis of the kidneys of these transgenic mice revealed that overexpression of wild-type WNK4 reduces the luminal surface area of the DCT, whereas overexpression of Q526E WNK4 increased the luminal surface area, as detected visually. Immunostaining indicated that expression levels of NCC were increased in Q526E WNK4 mice, but colocalization studies with segment markers indicated that NCC expression was still confined to the DCT. Little or no differences were observed in ROMK expression between wild-type and transgenic mice on normal or high K+ diets or in levels of ENaC expression.57 Importantly, interbreeding of Q526E WNK4 mice with NCC knockout mice resulted in complete amelioration of all of the defects observed in the Q526E WNK mice, suggesting that dysregulation of NCC is the key mechanism underlying FHHt.

One limitation regarding the studies of Lalioti et al57 is that they were based on overexpression of wild-type or expression of mutant WNK4 on a background of 2 wild-type WNK4 alleles. A more recent report described generation of a WNK4 D561A knockin mouse.58 Unlike the mice generated by Lalioti et al,57 which express mutant WNK4 on a wild-type WNK4 background, these mice express 1 mutant WNK4 allele and 1 wild-type allele, closely mimicking the human disease. The phenotype of these mice was remarkably similar to the phenotype of the WNK4 Q562E transgenic mice; the investigators also noted that the phenotypic effects were completely corrected by thiazide diuretics, like in humans.58 In addition, however, these investigators reported increased phosphorylation of the NCC, which may reflect enhanced NCC activity, independent of effects on trafficking (see Figure 3). Taken together, these results strongly suggest that mutations in WNK4 cause FHHt primarily by increasing NCC activity, abundance, and phosphorylation in the DCT.

Recently, Mayan et al59 detected increased NCC abundance in the urine of patients with WNK4 Q562E versus controls, providing further support for the relevance of these observations to humans.

One result of these studies has led to some confusion, however. As noted, wild-type WNK4 has been shown to inhibit NCC activity both in vitro and in animals. Some,31,50,53 although not all,51,60 investigators have reported that mutations of WNK4 abrogate its inhibitory activity. This has led some investigators to suggest that FHHt results from loss of WNK4-mediated NCC inhibition.31,50,53,58,61 Yet, the WNK4 Q562E transgenic animals express mutant WNK4 on a background that includes 2 wild-type WNK4 alleles57; the knockin animals express mutant WNK4 on a background of a single wild-type allele.58 In both situations, wild-type WNK4 is present and expressed within the kidney, suggesting that WNK4 mutations act as “gain-of-function” rather than “loss-of-function” mutations. Surprisingly, then, animals made transgenic for additional copies of wild-type WNK4 exhibit a phenotype that is opposite to FHHt and is strikingly similar to Gitelman’s syndrome.57 They have reduced NCC abundance and activity and hypokalemia; thus, as demonstrated using oocytes experiments, wild-type WNK4 reduces NCC activity and abundance at the plasma membrane. This indicates that wild-type and mutant WNK4 proteins exert opposite effects on NCC. Taken together the results clearly show that FHHt results from disordered regulation of NCC by WNK4, but a unifying explanation of the functional effects of wild-type and mutant WNK4 seems difficult to provide.

We recently reported evidence of WNK kinase effects that may help to resolve this conundrum. We found that WNK3 also participates in a WNK kinase signaling complex with WNK4.56 Rinehart et al47 showed that WNK3 stimulates NCC activity unopposed and NCC activity strongly stimulated by WNK4 Q562E had higher blood pressure than wild-type mice, whereas animals transgenic for WNK4 Q562E had higher blood pressure than wild-type mice. With regard to electrolyte balance, mice overexpressing the FHHt-causing mutant WNK4 displayed hyperkalemia to an extent similar to that seen in patients with FHHt. When challenged with a high K+ diet, Q526E WNK4 mice became more hyperkalemic, with clearly impaired K+ excretion. On a low K+ diet, wild-type WNK4 transgenic mice became hypokalemic relative to wild-type mice. Taken together, these data indicate that WNK4 plays a key role in regulating K+ balance, but the effects of WNK4 and mutant WNK4 on K+ balance are opposite.

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We also found that FHHt-mutant WNK4 Q562E loses its ability to inhibit WNK356; yet, as noted above, FHHt does not seem to result from loss of function. This raised the possibility that interactions between WNK4 and WNK3 might help to explain the FHHt phenotype. In support of this hypothesis, we found that WNK4 Q562E binds to and inhibits the effect of wild-type WNK4 on WKH3. Thus, WNK4 Q562E seems to act as a dominant-negative WNK4 modulator, leaving WNK3 activity unopposed and NCC activity strongly stim-
ulated. Although this model is derived from observations made in vitro and must be tested in more physiological systems, it is consistent with the opposing effects of wild-type and mutant WNK4 on NCC activity. Wild-type WNK4 suppresses NCC activity both directly and by inhibiting WNK3. FHHt mutant WNK4 not only loses the ability to inhibit WNK3 but also blocks the effects of the wild-type gene product, thereby leaving NCC activity enhanced. Figure 5 shows a simplified model of WNK3 and WNK4 interactions that could account for the phenotypes of the transgenic and knockin mice.

The WNK kinase signaling complex must play a role in normal electrolyte homeostasis, too. Although physiological regulators of WNK kinase activity and abundance are only beginning to be evaluated, it seems that dietary K\(^+\) loading increases WNK4 abundance\(^{46}\) and increases the ratio of KS-WNK1/WNK1.\(^{46,62,63}\) Figure 3 shows how both increased WNK4 abundance and an increased KS-WNK1/WNK1 ratio favor K\(^+\) secretion by shifting the DCT2 from transporting Na\(^+\) primarily with Cl\(^-\) (neutral) to transporting Na\(^+\) largely in exchange for K\(^+\) (electrogenic). First, the increase in WNK4 inhibits NCC activity directly, thereby enhancing electrogenic Na\(^+\) absorption relative to electroneutral Na\(^+\) reabsorption; second, the increased KS-WNK1 inhibits WNK1’s ability to block WNK4, thereby inhibiting NCC; third, the increased WNK4 inhibits WNK3; because WNK3 is a potent NCC stimulator, this effect will further suppress NCC activity. As discussed below, these effects on NCC do not exclude important and physiologically relevant effects of WNK kinases on other ion transport pathways but would tend to hyperpolarize the epithelium, favoring K\(^+\) secretion.

Wilson et al\(^{50}\) proposed that an unidentified physiological ligand switches WNK4 activity from inhibitory (to NCC) to stimulatory (to NCC), mimicking the human disease, FHHt. According to the proposed WNK signaling complex model, WNK3 is a WNK4 ligand that fulfills this prediction (see Figure 3). Conversely, the same investigators suggested that an unidentified physiological ligand might switch WNK3 from stimulatory (to NCC) to inhibitory (to NCC).\(^{47}\) The WNK signaling complex model (Figure 3) suggests that WNK4 is a WNK3 ligand that fulfills this prediction.

**Mechanisms of Hyperkalemia in FHHt**

Hyperkalemia is a universal feature of FHHt and frequently develops before the onset of hypertension. Renal K\(^+\) excretion results primarily from K\(^+\) secretion along the DCT2 and CNT,\(^{32,64}\) as well as the cortical collecting duct. Potassium secretion is driven by the electrical gradient generated by Na\(^+\)-
reabsorption (via ENaC) across an electrically tight epithelium. Paracellular permeability characteristics of the distal tubule are determined largely by expression of claudins, proteins that act as selective barriers to ion movement and, therefore, maintain the transepithelial voltage. \(^6\) Potassium is secreted largely, although not exclusively, via ROMK (Kir1.1) channels. In addition to affecting NCC, WNK kinases might, therefore, modulate K\(^+\) excretion by interacting with ENaC, ROMK, or claudins, among other proteins. The effects of WNK kinases on each of these protein classes will, therefore, be discussed (these effects are illustrated in Figure 3).

WNK kinases have been shown to modulate ENaC activity in cells, in Xenopus oocytes, and in vivo. WNK1 increases ENaC activity by activating phosphatidylinositol 3-kinase, stimulating glucocorticoid-induced kinase 1 \(^6\)\(^6\)\(^6\)\(^7\) a well-established ENaC-regulatory factor. \(^6\) This effect depends on an intact WNK1 kinase domain but also requires an intact amino terminal domain (N-terminal of the kinase domain). Interestingly, KS-WNK1, which lacks both the kinase domain and the amino-terminal domain, has also been reported to stimulate ENaC, implying a different mechanism. \(^6\) In contrast to WNK1, WNK4 inhibits ENaC activity, an effect that is suppressed by stimulating glucocorticoid-induced kinase 1. \(^7\) FHHt-mutant WNK4 (WNK4 Q562E) does not inhibit ENaC activity when expressed in Xenopus oocytes, \(^7\) and mice expressing an FHHt-mutant WNK4 exhibit increased ENaC activity in the kidney and colon. \(^5\) Based on these results, and results discussed below, Ring et al.\(^7\) suggest that the FHHt phenotype may result, in part, from the coordinated upregulation of NCC and ENaC and downregulation of ROMK (see below). Three observations, however, argue that activation of ENaC, whereas probably present in WNK4, at least when the disease results from WNK4 mutations. First, as originally suggested by Wilson et al.\(^1\) WNK4 strongly inhibits ROMK activity in vitro through a kinase-independent mechanism. \(^6\)\(^7\) WNK4 reduces ROMK abundance at the plasma membrane, as it does with NCC. Yet, the effect of WNK4 on ROMK is dynamin dependent and involves clathrin-mediated endocytosis, \(^7\) whereas the effect of WNK4 on NCC does not. Studies in Xenopus oocytes and HEK-293 cells have shown that WNK1 also inhibits ROMK activity. Two groups reported that the effect depends on intact kinase activity, \(^6\)\(^2\)\(^6\) whereas another found that a kinase-dead WNK1 mutant was still effective. \(^7\) Time course studies of the ROMK plasma membrane expression suggest that WNK1 increases endocytosis of ROMK in a dynamin-dependent manner, \(^7\) an effect involving interactions with the scaffolding protein intersectin. \(^7\)\(^4\) KS-WNK1 has no direct effect on ROMK activity but blocks the effects of WNK1 on ROMK. \(^6\)\(^2\)\(^6\) KS-WNK1 therefore indirectly activates ROMK and inhibits NCC.

Kahle et al.\(^2\) reported that FHHt-causing mutant WNK4 inhibits ROMK more actively than does wild-type WNK4 and suggested that hyperkalemia in FHHt could result from a reduced abundance of K channels at the plasma membrane. \(^7\) As with the observations regarding ENaC, however, observations from patients and animal models argue that reduced K conductance is not a primary cause of hyperkalemia in FHHt, at least when the disease results from WNK4 mutations. First, patients with FHHt can excrete normal amounts of potassium when nonchloride salts of Na\(^+\) are infused. \(^7\) This indicates that the potassium secretory apparatus is intact and suggests that reduced K\(^+\) secretion results from a reduced transepithelial voltage in the aldosterone-sensitive distal nephron. Second, animals transgenic for mutant WNK4 do not exhibit any apparent changes in ROMK abundance. \(^5\)\(^7\) Third, treatment with thiazides, which corrects the K\(^+\) secretory abnormalities, \(^5\)\(^7\) would not be expected to correct a defect in ROMK. It should be noted, however, that inhibition of NaCl reabsorption by thiazides would be expected to increase distal flow and could stimulate K secretion via structurally unique maxi-K channels. \(^7\) Yet, urinary Na\(^+\) and water excretion during chronic thiazide treatment are not different from baseline, despite persistent amelioration of hyperkalemia in FHHt. \(^7\) This observation suggests that the primary effects of thiazides to correct K balance do not require increases in urinary Na\(^+\) or water excretion.

Based on the observations concerning the effect of nonchloride sodium salts on K\(^+\) secretion in FHHt, Schambelan et al.\(^8\) proposed that FHHt results from a “chloride shunt” in the distal nephron. According to this model, increased chloride permeability in the distal nephron depolarizes the transepithelial voltage, which is oriented with the lumen negative relative to blood, such an effect would increase Na\(^+\) reabsorption and decrease K\(^+\) secretion, the former contributing to hypertension and the latter to hyperkalemia. The observation that WNK4 colocalizes with tight junctions \(^1\) suggested that WNK kinases might regulate paracellular chloride permeability and seemed consistent with the chloride shunt hypothesis. In support of this hypothesis, one group showed that WNK4 affects paracellular chloride permeability when overexpressed in cultured cells. \(^7\) Two groups also reported that FHHt-mutant WNK4 increased chloride permeability relative to Na\(^+\) permeability. \(^7\)\(^7\) This effect of WNK4 was reported to require WNK4 catalytic activity, because a kinase-inactive WNK4 did not affect paracellular chloride permeability \(^7\) and because WNK4 was reported to phosphorylate claudins 1 to 4. \(^7\) Recently, overexpression of L-WNK1 was reported to exert similar effects on chloride permeability. \(^7\) A relative increase in paracellular chloride permeability in response to mutant WNK4 would seem consistent with the chloride shunt model. Yet, as with effects of ENaC and ROMK, the ability of thiazide diuretics to correct the hyperkalemia in FHHt does not seem consistent with a predominant effect on paracellular processes. Thiazide diuretics do affect Cl\(^-\) trans-
port but have never been reported to alter paracellular processes. Furthermore, direct measurement of paracellular permeability from collecting ducts of mice with WNK4 D561A knocked-in show no difference from wild-type collecting ducts.58

Although the data discussed do not support a primary role for altered ENaC, ROMK, or claudin activity in the pathogenesis of FHH induced by WNK4 mutations, this does not mean that the effects of WNK kinases on transport proteins other than NCC do not contribute importantly to electrolyte homeostasis. Most of the described effects of WNK kinases on these transport pathways are consistent with a physiologically important role in regulating Na⁺, Cl⁻, and K⁺ transport under normal conditions. As described above, the unique effects of WNK4 mutations on NCC may, therefore, result from alterations in the WNK signaling complex that do not modulate ion transport by other pathways. A full discussion of such possibilities is beyond the scope of this review.

The Aldosterone Paradox
The FHH phenotype indicates that WNK kinases lie at a critical physiological control point that regulates the balance between NaCl absorption and K⁺ secretion, thereby modulating aldosterone’s actions to regulate blood pressure, on the one hand, and K⁺ balance, on the other. This suggests that consideration of WNK kinases might help answer a persistent question concerning the actions of aldosterone. Aldosterone is secreted in response to 2, sometimes independent, stimuli: extracellular fluid volume depletion and hyperkalemia. In the case of extracellular fluid volume depletion, aldosterone stimulates Na⁺ reabsorption (largely with chloride), whereas in hyperkalemia, aldosterone promotes K⁺ secretion.79 The ability of a single hormone to exert different effects has been documented by Vasuvaatukul et al79 and Halperin and Kamel80 and termed the “aldosterone paradox,” but a mechanistic explanation has not been apparent. Recently, it has been suggested that KS-WNK1 plays a key role.46,55,62,63 Evidence for this comes from studies examining the regulation of WNK kinase expression by dietary electrolyte manipulation46,55,62,63 and by aldosterone.46

The balance between electroneutral Na⁺ reabsorption and electrogenic Na⁺ reabsorption plays a pivotal role in this model. Under normal conditions, NCC mediates electroneutral NaCl reabsorption in the DCT1 and, to a lesser extent, the DCT2; in later segments, including the DCT2, ENaC activity generates a lumen-negative voltage, which drives K⁺ secretion. When aldosterone secretion is induced by dietary K⁺ loading, KS-WNK1, and probably WNK4, are induced as well.46,55,62,63 As discussed above, both KS-WNK1 and WNK4 inhibit NCC trafficking and favor electrogenic Na⁺ reabsorption (Figure 3). Along with direct effects of aldosterone on ENaC, the balance of Na⁺ reabsorption along the DCT2 shifts from electroneutral to electrogenic, increasing the transepithelial voltage and favoring K⁺ secretion. Effects of WNK kinases on ENaC, ROMK, and even claudins are likely to contribute additionally to these effects (see Figure 3). In contrast, during hypovolemia (low NaCl intake), aldosterone secretion is increased, but KS-WNK1 and WNK4 expression are not increased and may even be reduced.46

These changes activate the NCC, favoring electroneutral over electrogenic Na⁺ transport, thereby depolarizing the transmembrane voltage. In more distal segments that lack NCC, aldosterone still stimulates ENaC activity, which drives some K⁺ secretion, but the effect favors balanced NaCl reabsorption with Na⁺/K⁺ exchange.

Summary
Information about the roles of WNK kinases in normal physiology and in the pathogenesis of FHH has accumulated rapidly. The pathogenesis of FHH that results from mutations in WNK4 is currently being solved, although important questions remain. The disease involves activation of NCC along the DCT, probably because mutant WNK4 exerts a dominant-negative effect on wild-type WNK4. This permits unrelieved activation of WNK3. In contrast, the mechanisms that underlie FHH caused by WNK1 mutations are not as clear but may also involve a dominant-negative effect, involving KS-WNK1. A decrease in the KS-WNK1/WNK1 ratio would activate NCC and inhibit ROMK. Whether these changes account, by themselves or with other processes, for the disease phenotype awaits experimental confirmation. Clearly, WNK kinases stand at the crossroads of renal Na⁺, K⁺, and Cl⁻ transport; as such, they are attractive targets for drug development.

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