The WNK Kinase Network Regulating Sodium, Potassium, and Blood Pressure

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In the last 10 years, a number of previously unrecognized kinases interacting in the distal nephron have been identified as playing important roles in sodium, potassium, and BP regulation. Among these are the WNKs (with no lysine = K), serine threonine kinases, which are atypical because the catalytic lysine crucial for binding to ATP is located in subdomain I instead of II. The WNK kinases were identified while searching for novel members of the mitogen-activated protein kinase family.1 The finding that mutations in WNK kinases cause the monogenetic disorder, familial hyperkalemic hypertension (FHHt, also known as pseudohypoaldosteronism type II or Gordon syndrome),2 sparked further interest in these regulatory proteins.3 Subsequent studies implicate WNK kinases in the direct or indirect regulation of the major sodium and potassium transporters in the distal nephron and in the pathogenesis of hypertension.

Based on the disease phenotype resulting from WNK kinase mutations, it is clear that WNKs play important roles in regulating ion channels and transporters. There is now abundant evidence, both in vitro and in vivo, that WNK kinases regulate the renal outer medullary potassium channel (ROMK),4 the sodium potassium chloride cotransporter type 2 (NKCC2),5 the sodium chloride cotransporter (NCC),6 and the epithelial sodium channel (ENaC) (Figure 1).7 WNK kinases modulate many other transport proteins as well.8

Four WNK kinases are expressed in the kidney, WNK1, kidney-specific WNK1 (KS-WNK1), WNK3, and WNK4, where they are most abundant along the aldosterone-sensitive distal nephron; this segment comprises the distal convoluted tubule (DCT), connecting tubule (CNT), and collecting duct (CD) (Figure 1).9 The protein structure of WNKs consists of a kinase domain, an autoinhibitory domain, an autophosphorylation site, coiled-coil domains, and proline-rich sequences.8 WNK kinases affect solute transporters by discrete, but probably related actions, which include modulating trafficking of proteins to or from the plasma membrane and modulating transport protein phosphorylation. Although effects mediated by phosphorylation events are important, most require intermediary kinases. Intermediary kinases include STE20/SPS1-related, proline alanine-rich kinase, and oxidative stress responsive protein type 1 (OSR1),10,11 two highly homologous kinases that are phosphorylated and activated by WNK kinases.

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SPAK and OSR1 act in the thick ascending limb (TAL) and DCT, where they phosphorylate NKCC2 and NCC (Figure 1).10,11 Additionally, WNKs interact with serum and glucocorticoid-inducible kinase 1 (SGK1).12 SGK1 is an important kinase for the transduction of aldosterone’s effect and therefore acts in the aldosterone-sensitive distal nephron (Figure 1).13 We will first review briefly what is known about mechanisms by which WNKs regulate NaCl and K\(^+\) transport.

Second, we will review the physiologic and clinical relevance of renal WNKs and hypertension (Table 1). The focus of this review, therefore, is on the WNKs in the kidney, but it is important to emphasize that WNK kinases are not only expressed in the aldosterone-sensitive distal nephron (Figure 1).13

We will first review briefly what is known about mechanisms by which WNKs regulate NaCl and K\(^+\) transport. Second, we will review the physiologic and clinical relevance of renal WNKs and hypertension (Table 1). The focus of this review, therefore, is on the WNKs in the kidney, but it is important to emphasize that WNK kinases are not only expressed in the kidney, but also in brain, heart, lung, bone, testis, and the gastrointestinal tract. In these organs, a role for WNK kinases has been implicated for diseases as diverse as neuropathy, autism, cancer, and osteoporosis.8

Table 1. Potential clinical relevance of WNK kinases in nephrology

<table>
<thead>
<tr>
<th>Potential clinical relevance</th>
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<tr>
<td>Mutations in WNK1 or WNK4 cause familial hyperkalemic hypertension</td>
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<tr>
<td>Salt retention caused by aldosterone or angiotensin II is mediated via WNK kinases</td>
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<tr>
<td>Polymorphisms in the WNK1 and WNK4 genes are associated with hypertension</td>
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<tr>
<td>A low potassium diet may predispose to hypertension because it increases the WNK1/KS-WNK1 ratio leading to more salt reabsorption</td>
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<tr>
<td>WNK4 mediates the antinatriuretic effects of insulin in diabetes mellitus</td>
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<tr>
<td>The WNKs are promising candidates for development of new anti-hypertensive drugs</td>
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brane. We will examine the roles of trafficking and phosphorylation in the regulation of NKCC2, NCC, and ROMK. WNKs also seem to contribute to the regulation of ENaC primarily through SGK1,7,21 but the data are limited, and this subject is therefore not covered here.22

Regulation of the NCC
A great deal of attention has been focused on WNK regulation of NCC, because the clinical phenotype of FHHt is thiazide-sensitive. As shown in Figure 2, WNK4 is believed to inhibit NCC activity by reducing its plasma membrane abundance6; several investigators have also observed that WNK4 reduces the steady-state abundance of NCC.23 WNK4 does not seem to interfere with the endocytic retrieval of NCC, because studies in mammalian cells show that plasma membrane abundance of NCC is unaffected by wild-type or mutant dynamin, a GTPase responsible for clathrin-mediated endocytosis.24,25 Rather, WNK4 seems to affect the forward trafficking pathway by diverting post-Golgi NCC to lysosomal degradation, thereby preventing delivery to the plasma membrane (Figure 2).23 This concept was derived from cell studies in which brefeldin A was used to inhibit forward trafficking of NCC to the plasma membrane; brefeldin A was then washed out, and WNK4 was shown to reduce the rate of NCC recovery at the plasma membrane.26 Another protein involved in causing this detour of NCC to the lysosomes is sortilin, a receptor in the Golgi complex that helps routing of proteins to lysosomes.27 Further evidence for the WNK4-induced degradation of NCC in lysosomes was obtained by showing the reversal of this process by bafilomycin A1, which disturbs lysosomal function by inhibiting the vacuolar H+-ATPase.24

There is less information about how other WNK kinases affect protein trafficking. In contrast with an initial model that postulated that WNK1 modulates NCC only through WNK4, recent evidence suggested that WNK1 regulates trafficking by facilitating the final steps of NCC insertion into the plasma membrane by interacting with the SNARE protein STX-3.28 The exact role of WNK3 in NCC regulation remains elusive, but oocyte and cell studies showed that WNK3 is a positive regulator of NCC and that the net effect on NCC is determined by antagonism between WNK3 and WNK4.5,15 When expressed in *Xenopus* oocytes, the effects of WNK3 to increase NCC activity can be dissociated from effects on phosphorylation.29

Besides trafficking, NCC is regulated by phosphorylation. A number of recent studies showed that both plasma membrane abundance and phosphorylation of NCC are increased by aldosterone,30–32 angiotensin II,30,32,33 and, surprisingly, vasopressin.34,35 SGK1, WNKs, and/or SPAK are identified as the main intracellular mediators of these receptor–transporter cascades.30–33,35 Although phosphorylation and trafficking can be dissociated *in vitro*, it remains to be determined whether trafficking and phosphorylation can occur independently *in vivo* and what their relative contributions are in the regulation of NCC.

Regulation of the NKCC2
NKCC2, the major sodium transporter in the TAL, is an interesting example of how trafficking and phosphorylation act in concert to regulate transporter activity. Using *Xenopus* oocytes, Giménez and Forbush18 showed that, under isotonic and hypotonic conditions, NKCC2 retained 50% of its activity in the absence of phosphorylation of three important threonines (99, 104, 117 in the rabbit sequence). Phosphorylation of these three residues, however, was necessary to stimulate NKCC2 activity during hypertonicity. Also in oocytes, Ponce-Coria *et al.*18 found that the activation of NKCC2 by

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**Figure 2.** Model of NCC regulation by WNK kinases. NCC is trafficked as a monomer from the cytosol to the apical plasma membrane to become an inactive dimer (lower half of figure). Activation of the NCC dimer is achieved by phosphorylation through SPAK, stimulating NaCl transport (left). This process is regulated by WNK kinases. WNK4 (green symbol) inhibits the trafficking step of NCC by diverting it to the lysosome, a process that is mediated by sortilin and adaptin 3 (AP3). Conversely, WNK3 (red symbol) stimulates trafficking. WNK3 and WNK4 inhibit each other’s activities. WNK4 is inhibited by WNK1 (red symbol), which in turn is inhibited by KS-WNK1 (blue and red symbol). WNK1 and WNK3 are also thought to influence the activity of SPAK, thereby controlling the phosphorylation and thus activation step of NCC. At present, it is unknown how the endocytic retrieval of NCC is regulated (question mark symbol). See text for details and abbreviations.
Regulation of the ROMK
WNK kinases also modulate ROMK by affecting clathrin-mediated endocytosis. The proof for this mainly comes from a study in mammalian cells by He et al., showing that WNK1 and WNK4 interact with the endocytic scaffold protein intersectin. They showed that the proline-rich motifs of WNK1 and WNK4 bind to the SH3 domains of intersectin. This allows

Table 2. Overview of phenotypes of genetically modified mouse models targeting WNK kinases or associated proteins

<table>
<thead>
<tr>
<th>Mouse Model</th>
<th>Phenotype</th>
<th>Reference</th>
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| Salt-sensitive hypotensive models
  - NKCC2−/− (indomethacin rescued) | Bartter-like              | 54        |
  - SORLA−/−          | Bartter-like              | 37        |
  - NCC−/−            | Gitelman-like            | 56        |
  - transgenic (WNK4W7) | Gitelman-like            | 63        |
  - “hypomorphic” WNK4 | Gitelman-like            | 64        |
  - SPAK−/−           | Gitelman-like            | 66        |
  - SPAK243A/243A (kinase-dead knockin) | Gitelman-like | 67        |
  - SGK−/−            | Salt-sensitive hypotension, hyperkalemia | 60        |
| Salt-sensitive hypertensive models
  - transgenic (WNK4P44H) | FHHt-like | 63        |
  - WNK4D561A/K567A (knockin) | FHHt-like | 70        |
| No phenotype
  - transgenic (NCC) | No phenotype           | 71        |
  - KS-WNK1−/−       | No phenotype           | 73        |
  - collecting duct–specific ENaC−/− | No phenotype     | 59        |

See text for abbreviations of protein names and explanations of the phenotypes. FHHt, familial hyperkalemic hypertension.
Lessons from Animal Models

Although in vitro studies help unravel regulatory mechanisms, animal models, coupled with phenotypic analysis of human disease, provide physiologic context. Many lessons have been learned from the development of animals in which either the transport proteins themselves, or the novel regulatory factors, have been modified genetically (Table 2). We will discuss those genotypes that result in renal sodium loss and hypertension (salt-sensitive hypertension); the salt-sensitive hypertensive models are discussed in the next section. Although it is not the focus of this review, it is useful first to discuss animal models in which distal sodium transporters are deleted, because they provide comparison with effects of regulatory protein modification. These knockout animals typically resemble human diseases in which the human homologs of the transport proteins are defective. NKKCC2 dysfunction in humans is one cause of Bartter syndrome; NKCC2 knockout mice die of dehydration before weaning, unless rescued by indomethacin, in which case they exhibit a Bartter-like phenotype, with salt wasting, hypokalemia, and hypercalciuria. NCC dysfunction in humans causes Gitelman syndrome, with hypokalemic alkalosis, hypocalciuria, and magnesium wasting; NCC knockout animals exhibit a milder phenotype with hypomagnesemia and hypocalciuria, but no other electrolyte or BP abnormalities when consuming a normal diet. When challenged with a low salt diet, however, BP declines more in the NCC-null animals than in wild type; when challenged with a potassium-deficient diet, hypokalemia ensues. ENaC dysfunction also causes pseudohypoaldosteronism type I in humans with profound neonatal salt wasting; ENaC deletion causes a similar syndrome in animals when rescued from perinatal death, although ENaC deletion from the collecting duct alone does not cause a substantial phenotype, even when challenged with salt restriction, water deprivation, or potassium loading.

The transporter-knockout models discussed above provide comparative information to analyze effects of regulatory proteins. Several mouse models have been generated in which proteins known to regulate NCC have been modified genetically, including WNK4, SPAK, and SGK1 (Table 2). SGK1-null mice do not recapitulate the effects of ENaC deletion but do exhibit hyperaldosteronism, mild hyperkalemia, and salt-sensitive hypertension because of reduced ENaC activity. Under baseline conditions, NCC abundance is normal, but when stressed with a low salt diet, NCC does not increase as much in SGK1 knockout as in wild type, providing evidence for an effect of SGK1 on NCC in vivo.

Animals transgenic for wild-type WNK4 exhibit reduced abundance of NCC. The BP and urinary calcium excretion are low, compared with wild-type animals, and they have hypokalemia on low potassium diets, providing evidence that WNK4 exerts an inhibitory effect on NCC in vivo. Animals in which WNK4 has been deleted have not been reported, because it has been suggested that WNK4 deletion may be embryonic lethal. Instead, Ohita et al. generated WNK4 hypomorphic animals, by deleting one exon of the WNK4 gene outside...
the kinase domain. The animals exhibit salt-sensitive hypotension and a reduction in phosphorylated, but not total, NCC. SPAK and ROMK were unaltered, whereas the expression of all ENaC subunits was increased. The authors interpreted the results as indicating that wild-type WNK4 exhibits NCC-stimulating activity at baseline. This finding contrasts with the conclusions reached above, that WNK4 exhibits NCC-inhibiting activity. In view of the suggestion that it is the kinase activity of WNK4 that, through SPAK, stimulates NCC activity, it is unclear how deletion of a non-kinase WNK4 exon alters NCC activity; clearly, this is an area that requires further investigation.

The effects of SPAK in vivo have been analyzed using a knockout and knockin strategy, in which an essential T-loop threonine is mutated to alanine to prevent kinase activation. Resulting animals exhibit a Gitelman phenotype with reduced expression and phosphorylation of NCC. It is noteworthy that mice with absent or inactive SPAK but not NCC-null mice display a profoundly lower baseline BP. This suggests that SPAK’s regulation of NCC is not the only mechanism by which SPAK regulates BP. Indeed, SPAK-null mice also have impaired vasoconstriction, likely because SPAK phosphorylates NKCC1 in vascular smooth muscle cells. It is noteworthy that mice with absent or inactive SPAK but not NCC-null mice display a profoundly lower baseline BP. This suggests that SPAK’s regulation of NCC is not the only mechanism by which SPAK regulates BP. Indeed, SPAK-null mice also have impaired vasoconstriction, likely because SPAK phosphorylates NKCC1 in vascular smooth muscle cells. Still unresolved, however, is how SPAK can differentially regulate NKCC2 and NCC and how different manipulations of SPAK can either result in a Bartter or a Gitelman phenotype.

In summary, these in vivo studies showed that genetic inhibition of WNK4, SPAK, or SGK1 results in reduced NCC activity, leading to a reduced ability to retain sodium with hypotension, especially during salt depletion. The studies to date, however, are limited by the experimental approaches, leaving many questions unanswered.

CLINICAL RELEVANCE

Familial Hyperkalemic Hypertension

The most obvious clinical relevance of the WNK kinases is the insight in the molecular pathogenesis of FHHt. FHHt is a rare autosomal dominant disorder characterized by hypertension, hyperkalemia, hypercalciuria, and metabolic acidosis, all of which can be corrected with thiazide diuretics. FHHt can occur by intronic deletions, causing overexpression of wild-type WNK1 or by missense mutations causing mutant WNK4. Although the mutations in WNK4 and WNK1 lead to largely (although perhaps not completely) similar phenotypes, the mechanisms by which the mutations lead to disease may differ. Mice transgenic for genomic segments harboring mutant WNK4 faithfully recapitulate the FHHt phenotype described above (Table 2). The same is true for mutant WNK4 knockin mice in which increased total NCC, phosphorylation of NCC, SPAK, and OSR1 are observed. These observations suggest that WNK4 mutations cause the phenotype primarily, if not exclusively, by stimulating NCC activity. In preliminary work, however, we observe that mice transgenic for NCC do not reproduce the FHHt phenotype, suggesting the involvement of additional factors.

The model shown in Figure 2, derived largely from work in vitro, suggests that WNK1 mutations lead to FHHt by altering the KS-WNK1/WNK1 ratio, thereby affecting NCC. This led investigators to generate mice in which intron 1 of the WNK1 gene was deleted. Surprisingly, this deletion caused overexpression of both WNK1 and KS-WNK1 in the DCT and other renal segments, whereas KS-WNK1 was also overexpressed in other tissues. The phenotype of these mice has not been reported. The same group also generated KS-WNK1-null mice; these did show a two-fold increase in NCC expression, confirming that KS-WNK1 acts as a WNK kinase network inhibitor in vivo. However, neither systolic hypertension nor hyperkalemia was observed, again illustrating that additional effects are required for the development of the complete FHHt phenotype. Conversely, mice overexpressing the KS-WNK1 amino terminus display hypokalemia and increased ROMK abundance, as well as hypotension and a decrease in NCC abundance. These data provide strong support for the model, originally postulated by Subramanya et al., that KS-WNK1 acts as a dominant-negative inhibitor of WNK1 in vivo (Figure 2).

Essential Hypertension

The fact that hypertension is a major risk factor for cardiovascular and renal disease is well established. The true challenge is solving the hypertension paradox—more uncontrolled disease despite improved therapy. One contributor to this paradox could be that our understanding of molecular pathways that convey susceptibility to hypertension still lags behind therapeutic options. Interestingly, the majority of the identified hypertension susceptibility genes all point toward a role for the kidney, which is consistent with Guyton’s prediction. The WNKs are a promising new link to hypertension. Indeed, several population studies now identify single nucleotide polymorphisms and haplotypes in the WNK1 and WNK4 genes that are not only associated with BP variation but also with hypertension severity, salt sensitivity, thiazide sensitivity, and urine potassium excretion. Interestingly, the WNK1 and WNK4 single nucleotide polymorphisms with the strongest associations were either located in or near the sites of the FHHt mutations. Although the effects of variants in a single gene may be modest, one study showed that when variants in the genes for WNK1, α-adducin (a cytoskeleton protein that also influences Na⁺/K⁺-ATPase activity), and Nedd4–2 (which ubiquinates ENaC) were combined, a significant effect was found on renal salt handling, the BP response to saline and thiazides, and nocturnal systolic BP.

Except for polymorphisms, it would also be of interest to know whether heterozygosity for WNK, SPAK, or SGK kinases determines susceptibility to hypertension. The reverse was recently shown in the Framingham Heart Study population, where heterozygous inactivating mutations in NKCC2, NCC, and ROMK protected against hypertension. Similarly,
mice heterozygous for the WNK1 mutation show a marked reduction in BP without other apparent side effects. This latter finding makes the WNKs obvious candidates as drug targets, and several screening strategies are already making progress. Although the development of isoform-specific inhibitors may be a challenge, there is substantial evidence that the WNK substrate specificity is not uniform, despite highly homologous kinase domains.17

Hypertension in Diabetes Mellitus
Besides the effects of angiotensin II and aldosterone, the effect of insulin on sodium reabsorption in the distal tubule is also becoming clearer.90 Hyperinsulinemia is a prominent feature of insulin resistance, diabetes mellitus, and obesity. Insulin receptors are expressed in the proximal tubule, DCT, and CD.90 Song et al.91 showed that the rise in BP in rats on chronic insulin treatment is likely caused by enhanced sodium reabsorption by NCC and ENaC, because treatment with hydrochlorothiazide and amiloride results in increased natriuresis. Interestingly, insulin also reduces renal cortical WNK4 expression, which contributes to activation of NCC and ENaC.91 NCC, the β-subunit of ENaC, and Na⁺-K⁺-ATPase also upregulate in the obese Zucker rat, a model of insulin resistance and obesity.92 More recently, the obese Zucker rat was shown to be more sensitive to thiazides than their lean counterparts, with a greater natriuresis, kaliuresis, and drop in BP on thiazides.93 Reduced renal cortical expression of WNK4 was also observed in this model. In addition to the possible regulation of NCC by insulin through WNK4, SGK1 is an established mediator of the effect of insulin on ENaC.13 Surprisingly, down-regulation of insulin receptors also causes salt retention and hypertension, possibly through reduced renal nitric oxide production.94 Therefore, the contribution of insulin-induced anti-natriuresis relative to other anti-natriuretic factors in diabetes, such as higher plasma aldosterone levels, remains to be determined.95 Nevertheless, it is an interesting example of how the WNK kinase pathway may also play a role in acquired forms of hypertension.

CONCLUSIONS
Insights derived from the discovery of WNK kinases and the elucidation of their renal effects represent a new paradigm for the molecular physiology of renal sodium, potassium, and BP regulation. Despite startling progress in understanding their roles, however, many questions remain, including the precise mechanisms by which they regulate protein trafficking and phosphorylation, the exact pathogenesis of FHHT, and the role of WNKs in the development of hypertension. The current models, such as that shown in Figure 2, must be placed in the perspective of the methods used to obtain the data, which have often relied on cell systems. Although important, these studies should be complemented with physiologic studies in whole animals and genetic epidemiologic approaches in humans to establish an integrated model of this complex kinase network in health and disease.

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DISCLOSURES
None.

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