

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

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ABSTRACT

The relationship between renal salt handling and hypertension is intertwined historically. The discovery of WNK kinases (With No lysine = K) now offers new insight to this relationship because WNKs are a crucial molecular pathway connecting hormones such as angiotensin II and aldosterone to renal sodium and potassium transport. To fulfill this task, the WNKs also interact with other important kinases, including serum and glucocorticoid-regulated kinase 1, STE20/SPS1-related, proline alanine-rich kinase, and oxidative stress responsive protein type 1. Collectively, this kinase network regulates the activity of the major sodium and potassium transporters in the distal nephron, including thiazide-sensitive Na-Cl cotransporters and ROMK channels. Here we show how the WNKs modulate ion transport through two distinct regulatory pathways, trafficking and phosphorylation, and discuss the physiologic and clinical relevance of the WNKs in the kidney. This ranges from rare mutations in WNKs causing familial hyperkalemic hypertension to acquired forms of hypertension caused by salt sensitivity or diabetes mellitus. Although many questions remain unanswered, the WNKs hold promise for unraveling the link between salt and hypertension, potentially leading to more effective interventions to prevent cardiorenal damage.

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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 (SPAK) 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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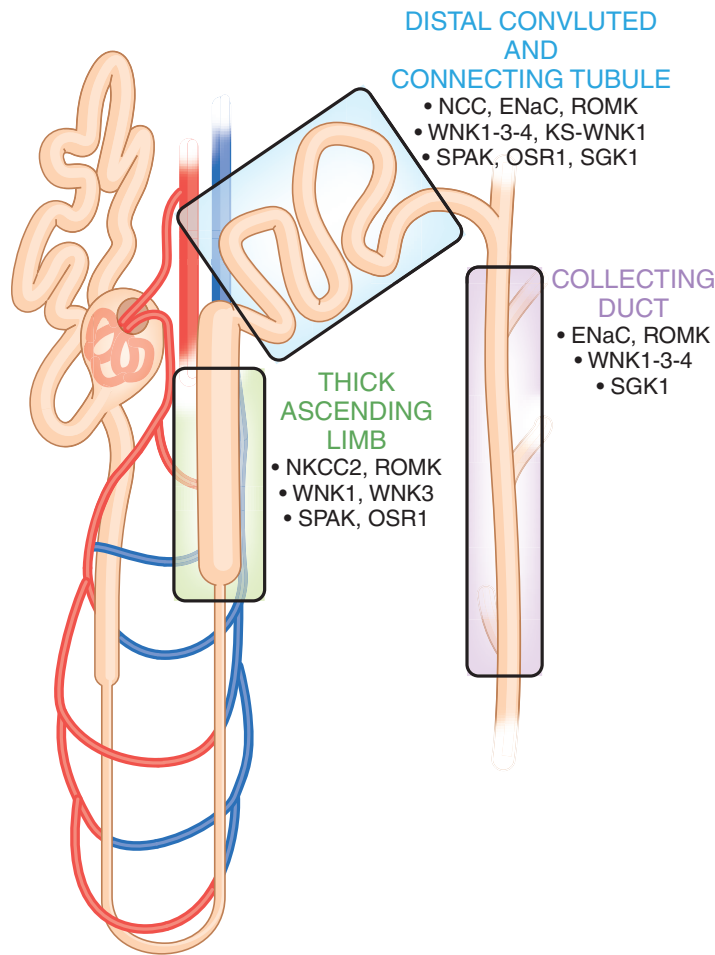


Figure 1. Expression of major sodium and potassium transport proteins in the distal nephron, including WNK kinases and associated regulatory proteins. See text for abbreviations.

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 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

WNK KINASES: TRAFFICKING AND PHOSPHORYLATION

As stated eloquently by Bradbury and Bridges,¹⁴ “membrane transport falls into two fundamental categories: alteration in transport activities (*i.e.*, transporter kinetics) of membrane resident proteins or alterations in the number of transport proteins per unit area.” The second category can again be divided into *de novo* synthesis of transport proteins, occurring in hours, and the rapid recruitment of preformed transporters residing in intracellular vesicles to the plasma membrane, occurring in minutes. The latter is often referred to as trafficking and constitutes a dynamic recycling process of endocytic retrieval and plasma membrane insertion of transport proteins. Phosphorylation, on the other hand, is one of the most important post-translational modifications that can activate or deactivate proteins through conformational changes. Because WNKs are kinases, it is not surprising that they modulate transporter function by affecting phosphorylation. It is unclear, however, if WNK kinases are capable of phosphorylating transporters directly.^{15,16} Instead, there is evidence that WNK1, WNK3, and WNK4 phosphorylate SPAK and OSR1, which in turn phosphorylate and activate NKCC2 and NCC.^{10,17–20} Both trafficking and phosphorylation are influenced by hormones to meet the requirements of the body and restore homeostasis. Although trafficking and phosphorylation are distinct and unique regulatory modalities, they are probably closely related under most physiologic conditions. At present, it is unknown whether WNK-regulated transport proteins are phosphorylated in the cytoplasm, thereby signaling movement to and insertion into the plasma membrane, or whether phosphorylation takes place once the protein is located in the plasma mem-

Table 1. Potential clinical relevance of WNK kinases in nephrology

Mutations in WNK1 or WNK4 cause familial hyperkalemic hypertension
Salt retention caused by aldosterone or angiotensin II is mediated via WNK kinases
Polymorphisms in the WNK1 and WNK4 genes are associated with hypertension
A low potassium diet may predispose to hypertension because it increases the WNK1/KS-WNK1 ratio leading to more salt reabsorption
WNK4 mediates the antinatriuretic effects of insulin in diabetes mellitus
The WNKs are promising candidates for development of new anti-hypertensive drugs

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.

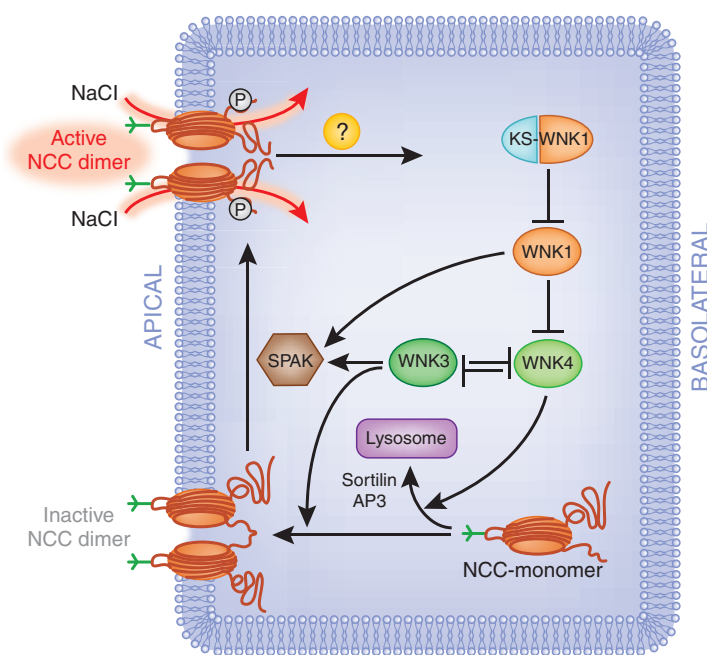


Figure 2. Model of NCC regulation by WNK kinases. NCC is trafficked as a monomer from the cytosol to the apical plasma membrane to become an inactive dimer (lower half of figure). Activation of the NCC dimer is achieved by phosphorylation through SPAK, stimulating NaCl transport (left). This process 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 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 Forbush³⁶ 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

low intracellular chloride also required phosphorylation of the three highly conserved threonines. In addition, they found that phosphorylation of NKCC2 also requires an interaction between WNK3 and SPAK, because elimination of WNK3's SPAK-binding motif, or catalytically inactive WNK3, prevents the activation of NKCC2. Based on these data, the investigators postulated WNK3 to be a chloride sensor. Recently, it was shown that NKCC2 phosphorylation also requires proper trafficking of SPAK by a trafficking protein called sorting protein-related receptor with A-type repeats (SORLA).³⁷ SORLA is expressed in the TAL, and SORLA-deficient mice exhibit missorting of SPAK and an inability to phosphorylate NKCC2. The mice had a mild Bartter-like phenotype with lower BP, higher aldosterone, kaliuresis, and calciuresis, and an inability to conserve sodium during water deprivation (Table 2).

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.,³⁸ 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

the formation of a complex consisting of ROMK interacting with WNK4 and WNK1–WNK4 interacting with intersectin.³⁹ Intersectin is a crucial protein in orchestrating endocytosis by recruiting dynamin and synaptojanin, eventually leading to endocytosis in clathrin-coated vesicles. Recently, it was shown that the recruitment of ROMK to clathrin-coated vesicles is mediated by a protein called clathrin adaptor molecule in autosomal recessive hypercholesterolemia (ARH).⁴⁰ Functional data to support a role for ARH in ROMK and potassium regulation include an inverse correlation between ARH and ROMK expression during potassium depletion and the inability of ARH-deficient mice to downregulate ROMK during potassium depletion.⁴⁰

PHYSIOLOGIC RELEVANCE

The Aldosterone Paradox

Contraction of the extracellular fluid volume is a threat to survival, and as a consequence, mammalian kidneys conserve sodium avidly. Hyperkalemia is equally threatening, and as a consequence, kidneys can secrete potassium rapidly. The ability of kidneys to conserve sodium and excrete potassium may reflect dietary composition in Paleolithic times; during that period, diets were so-

dium deplete but potassium replete.^{41,42} Aldosterone plays a central role in renal control of both sodium chloride reabsorption and potassium secretion. Indeed, major renal sodium and potassium transporters are regulated by aldosterone, including NCC,⁴³ ENaC,⁴⁴ and ROMK.^{45,46} However, the challenges of volume depletion and potassium overload are often dissociated, and aldosterone secretion is stimulated by either angiotensin II (in states of extracellular fluid volume depletion) or serum K⁺ concentration (in states of K⁺ excess; Figure 3). When aldosterone is secreted in response to angiotensin II, its primary effect is NaCl retentive; when it is stimulated in response to hyperkalemia, its primary effect is kaliuretic.⁴⁷ The observation that aldosterone can serve distinct functions, depending on the secretory stimulus, has been termed the aldosterone paradox.⁴² Explanations hinge on the balance between NaCl transport and Na⁺/K⁺ exchange, but until recently, mechanisms have remained elusive.

There are likely to be several factors involved in mediating the discrepant effects of aldosterone. As shown in Figure 3, angiotensin II, in the presence of aldosterone, shifts the balance of Na⁺ reabsorption proximally. This proximal shift favors NaCl reabsorption. In contrast, aldosterone secretion in a setting of low angiotensin II (as in hyperkalemia) shifts Na⁺ reabsorption distally, favoring K⁺ secretion. Many factors contribute to these effects, but the WNK kinase network seems to play a role, acting as a molecular switch in the aldosterone-sensitive distal nephron (Figure 3).⁴⁸ The evidence for a switching effect includes the observation that FHHT is a disease in which the ability to dissociate the kaliuretic from the NaCl retentive effects is lost.^{49,50} This model, suggesting that WNK regulation participates in determining the nature of aldosterone action, is also supported by the observation that dietary K⁺ intake has substantial effects on WNK kinase abundance. Both the KS-WNK1/WNK1 ratio and the abundance of WNK4 increase on high potassium diets (hyperkalemia), but not on a low sodium diet (hypovolemia).^{51,52} Ac-

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

Mouse Model	Phenotype	Reference
Salt-sensitive hypotensive models		
NKCC2 ^{-/-} (indomethacin rescued)	Bartter-like	54
SORLA ^{-/-}	Bartter-like	37
NCC ^{-/-}	Gitelman-like	56
transgenic (WNK4 ^{WT})	Gitelman-like	63
"hypomorphic" WNK4	Gitelman-like	64
SPAK ^{-/-}	Gitelman-like	66
SPAK ^{243A/243A} (kinase-dead knockin)	Gitelman-like	67
SGK ^{-/-}	Salt-sensitive hypotension, hyperkalemia	60
Salt-sensitive hypertensive models		
transgenic (WNK4 ^{PHAlI})	FHHT-like	63
WNK4 ^{D561A/+} (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.

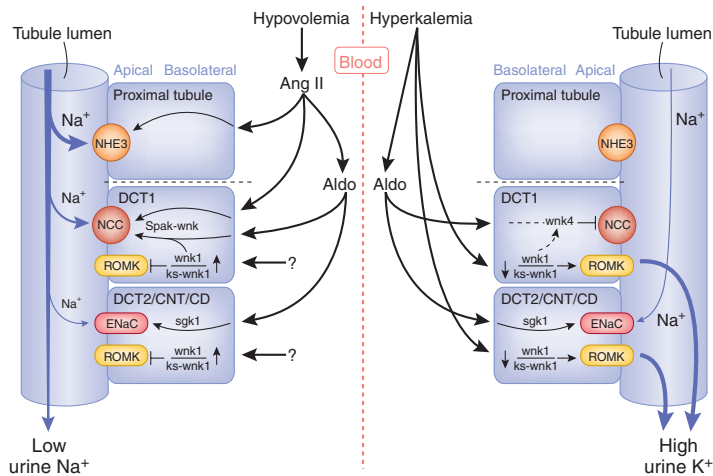


Figure 3. Model of the “aldosterone paradox.” Two pathophysiological settings are depicted: hypovolemia (left) and hyperkalemia (right). Aldosterone acts as a sodium retaining hormone during hypovolemia, leading to a low urinary Na^+ excretion (left). Conversely, aldosterone acts as a K^+ -secreting hormone during hyperkalemia, leading to a high urinary K^+ excretion (right). Hypovolemia stimulates angiotensin II (Ang II), which in turn increases aldosterone (Aldo). Both contribute to renal Na^+ retention. Ang II stimulates Na^+ transport in the proximal tubule by activating the NHE3. Ang II also increases the activity of the NCC in the early DCT1. Aldo activates both NCC and the ENaC in the late DCT (DCT2), CNT, and CD. Note that because Na^+ transport is stimulated at three locations, the distal delivery of Na^+ decreases, contributing to the low Na^+ excretion. The effects of Ang II and Aldo are primarily mediated via a WNK–SPAK pathway, whereas the effects of Aldo on ENaC primarily involves SGK1. Unknown factors increase the WNK1–KS–WNK1 ratio, leading to inhibition of the ROMK, helping to conserve potassium during hypovolemia. In the setting of hyperkalemia, the opposite occurs, because direct effects of a high serum K^+ level decrease the WNK1–KS–WNK1 ratio (right). This leads to an activation of ROMK, stimulating potassium secretion. The lower WNK1–KS–WNK1 ratio also increases WNK4, preventing Aldo from activating NCC (dashed line). However, Aldo is still capable of activating ENaC, which stimulates Na^+ exchange for K^+ in the collecting duct.

According to the current model, the increase in KS–WNK1 and WNK4 would favor electrogenic transport with potassium secretion (Figure 3).⁵³ The WNK kinase network is also modulated by angiotensin II, which is elevated when aldosterone secretion is stimulated by hypovolemia but not when it is stimulated by hyperkalemia. Indeed, there is accumulating evidence that angiotensin II, through WNK4 and SPAK, activates NCC, favoring NaCl reabsorption during hypovolemia.^{32,33}

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 hypotension (salt-sensitive hypotension); 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. NKCC2 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.⁵⁴ 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.^{55,56} When challenged with a low salt diet, however, BP declines more in the NCC-null animals than in wild type⁵⁶; when challenged with a potassium-deficient diet, hypokalemia ensues.⁵⁷ ENaC dysfunction also causes pseudohypoaldosteronism type 1 in humans with profound neonatal salt wasting⁵⁸; 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.⁵⁹

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 hypotension because of reduced ENaC activity.⁶⁰ 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,^{61,62} 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,⁶³ 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.⁶⁴ Instead, Ohta et al.⁶⁴ 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.⁶⁴ 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.^{6,65} 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 knockout⁶⁶ and knockin strategy, in which an essential T-loop threonine is mutated to alanine to prevent kinase activation.⁶⁷ Resulting animals exhibit a Gitelman phenotype with reduced expression and phosphorylation of NCC.^{66,67} It is noteworthy that mice with absent or inactive SPAK but not NCC-null mice display a profoundly lower baseline BP.^{56,66,67} 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.⁶⁶ Still unresolved, however, is how SPAK can differentially regulate NKCC2 and NCC and how different manipulations of SPAK can either result in a Bartter³⁷ or a Gitelman phenotype.^{66,67}

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 mo-

lecular 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.⁶⁸ 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 completely⁶⁹) 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).⁶³ The same is true for mutant WNK4 knockin mice in which increased total NCC, phosphorylation of NCC, SPAK, and OSR1 are observed.⁷⁰ 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.⁷¹

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.⁷² 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*.⁷³ However, neither systolic hypertension nor hyperkalemia was observed, again illustrating that additional effects are required for the development of the complete FHHt phenotype.⁷³ Conversely, mice overexpressing the KS-WNK1 amino terminus display hypokalemia and increased ROMK abundance, as well as hypotension and a

decrease in NCC abundance.⁷⁴ These data provide strong support for the model, originally postulated by Subramanya *et al.*,⁷⁵ 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.⁷⁶ 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,⁷⁷ which is consistent with Guyton's prediction.⁷⁸ The WNKs are a promising new link to hypertension.^{65,79} Indeed, several population studies now identify single nucleotide polymorphisms and haplotypes in the *WNK1* and *WNK4* genes that are not only associated with BP variation⁸⁰ but also with hypertension severity,⁸¹ salt sensitivity,⁸² thiazide sensitivity,⁸³ and urine potassium excretion.⁸⁴ Interestingly, the WNK1 and WNK4 single nucleotide polymorphisms with the strongest associations were either located in or near the sites of the FHHt mutations.^{84,85} 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 ubiquitinates ENaC) were combined, a significant effect was found on renal salt handling, the BP response to saline and thiazides, and nocturnal systolic BP.⁸⁶

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.⁸⁷ Similarly,

mice heterozygous for the WNK1 mutation show a marked reduction in BP without other apparent side effects.⁸⁸ This latter finding makes the WNKs obvious candidates as drug targets, and several screening strategies are already making progress.⁸⁹ 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 $\text{Na}^+\text{-K}^+\text{-ATPase}$ 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, downregulation 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 path-

way 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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