Role of WNK kinases in regulating tubular salt and potassium transport and in the development of hypertension

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Gamba, Gerardo. Role of WNK kinases in regulating tubular salt and potassium transport and in the development of hypertension. Am J Physiol Renal Physiol 288: F245–F252, 2005; doi:10.1152/ajprenal.00311.2004.—A recently discovered family of protein kinases is responsible for an autosomal-dominant disease known as Gordon’s syndrome or pseudohypoaldosteronism type II (PHA-II) that features hyperkalemia and hyperchloremic metabolic acidosis, accompanied by hypertension and hypercalciuria. Four genes have been described in this kinase family, which has been named WNK, due to the absence of a key lysine in kinase subdomain II (with no K kinases). Two of these genes, WNK1 and WNK4, located in human chromosomes 12 and 17, respectively, are responsible for PHA-II. Immunohistochemical analysis revealed that WNK1 and WNK4 are predominantly expressed in the distal convoluted tubule and collecting duct. The physiological studies have shown that WNK4 downregulates the activity of ion transport pathways expressed in these nephron segments, such as the apical thiazide-sensitive Na⁺Cl⁻ cotransporter and apical secretory K⁺ channel ROMK, as well as upregulates paracellular chloride transport and phosphorylation of tight junction proteins such as claudins. In addition, WNK4 downregulates other Cl⁻ influx pathways such as the basolateral Na⁺-K⁺-2Cl⁻ cotransporter and Cl⁻/HCO₃⁻ exchanger. WNK4 mutations behave as a loss of function for the Na⁺-Cl⁻ cotransporter and a gain of function when it comes to ROMK and claudins. These dual effects of WNK4 mutations fit with proposed mechanisms for developing electrolyte abnormalities and hypertension in PHA-II and point to WNK4 as a multifunctional regulator of diverse ion transporters.

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decrease in aldosterone levels usually seen in volume overload states is not present. Because K\textsuperscript+ is a strong stimulus for aldosterone secretion (12), normal-to-high aldosterone levels in the setting of volume overload in PHA-II patients could be explained by hyperkalemia, which is a major feature of the disease. No clinical study has addressed this issue in PHA-II patients.

Soon after PHA-II was described, it was observed that thiazide diuretics were very effective to treat not only elevated blood pressure but also all metabolic features of the disease (14). The high sensitivity to thiazides was described as the most consistent feature of this syndrome, because virtually all patients responded to treatment with these diuretics (13). In recent studies by Mayan et al. (25, 26) in 18 affected and 16 unaffected members of a single kindred, high sensitivity to thiazides was demonstrated. It was observed that reduction of blood pressure with a small dose of hydrochlorothiazide was about six to seven times higher than the reduction expected for an essential hypertensive population. The investigators observed that in contrast to nonaffected relatives, in addition to hypertension and hyperkalemic metabolic acidosis, PHA-II patients also exhibited hypercalciuria and a significant decrease in bone mineral density (BMD). Supporting the hypothesis that volume overload could be due to increased activity of TSC, all of these data reveal that PHA-II is the mirror image of Gitelman’s disease, which is due to inactivating mutations of the $SLC12A3$ gene that encodes for TSC and features hypertension, hypokalemia, metabolic alkalosis, hypocalciuria, and increased BMD (7). Thus it has been suggested that increased activity of TSC in the distal convoluted tubule (DCT) could potentially explain the development of PHA-II. However, no significant linkage was observed between PHA-II and the $SLC12A3$ locus on human chromosome 16 (37). An alternative possibility to explain the increased activity of the DCT resulting in volume overload, thiazide sensitivity, hypokalemia, and hypercalciuria would be if a gene defect in PHA-II results in DCT hypertrophy and/or hyperplasia. An example of this possibility is the syndrome known as apparent excess of mineralocorticoid (SAME), in which inactivating mutations of the enzyme 11β-hydroxysteroid dehydrogenase type 2 (29, 47) allow cortisol to extend its half-life and illegitimately occupy the mineralocorticoid receptor, resulting in arterial hypertension. In this disease, the excess of mineralocorticoid activity induced by cortisol is associated with hypertrophy and hyperplasia of the DCT (20), leading to overactivity of this nephron segment, including the development of hypercalciuria and nephrocalcinosis (28).

The chloride shunt hypothesis was proposed by Schambelan et al. (36) after they performed a careful metabolic study of a 23-yr-old male patient. Because aldosterone levels are normal to high in this illness, it was suggested that PHA-II represents a kind of mineralocorticoid-resistant state and the name pseudohypoaldosteronism type II was proposed. In this regard, however, most patients in whom the response to endogenous or exogenous mineralocorticoid administration has been studied exhibited no evidence of resistance (13), suggesting that increased aldosterone levels are the consequence of hyperkalemia rather than resistance to mineralocorticoid effects. Schambelan et al. (36) also observed in the patient studied that the urinary K\textsuperscript+ excretion rate was increased by intravenous infusion of sodium sulfate, but not of sodium chloride. Based on these observations, they proposed that the primary abnormality in PHA-II could be an increased rate of chloride reabsorption by renal tubules, which, in turn, diminished the physiological lumen-negative transtubular electrical potential difference, thus reducing the electrical driving force that in distal nephron is a major determinant of K\textsuperscript+ secretion. When luminal chloride was replaced with a poorly reabsorbable anion such as sulfate, the presence of sulfate in tubular fluid would eliminate the abnormal shunting effect created by increased chloride reabsorption, restoring the lumen-negative electrical difference that drives K\textsuperscript+ secretion. In this case, increased paracellular reabsorption of chloride would be followed by increased sodium reabsorption in the collecting duct (CD) and thus a volume-overload state.

SOME FORMS OF PHA-II ARE DUE TO MUTATIONS IN WNK KINASES

Genomic analysis of kindreds with PHA-II revealed no linkage with $SLC12A3$ encoding TSC (37) but showed significant association with several regions along the human genome. Mansfield et al. (23) performed a genomic-wide linkage search of the human genome in eight families with PHA-II. Six families exhibited a log of the odds (LOD) score of 8.1 for linkage with chromosome 1q31–42, and two families had a similar score for linkage with chromosome 17p11-q21. Disse-Nicodeme et al. (9) studied a large French pedigree that exhibited a strong linkage (LOD score of 6.12) with the chromosome 12p13 region. In addition, Disse-Nicodeme et al. (10) have also shown that at least one other locus is associated with the disease in two kindreds in which linkage with the identified loci in chromosomes 1, 12, and 17, as well as with $SLC12A3$ was negative. These studies indicated that at least four genes are capable of producing a similar type of PHA-II and that the $SLC12A3$ gene is not one of them. Today, two of the four genes have been uncovered by Wilson et al. (45). In this study, it was first demonstrated that a kindred with significant linkage with chromosome 12p13 cosegregated with a novel 41-kb genomic deletion located within the FIRST large intron of the human ortholog of a recently described serine-threonine kinase in the rat known as WNK1 (48). Then, other chromosome 12-linked kindreds were shown to cosegregate with a 21-kb deletion in the same intronic region of WNK1. No mutations or deletions in the human WNK1 coding sequence were detected in these families. Thus it was proposed that PHA-II in a kindred’s linkage to the chromosome 12p13 region is due to genomic deletions of the first intron of WNK1, resulting in increased expression of this kinase. Indeed, analysis of transcript levels of WNK1 in leukocytes from affected and unaffected members of these families revealed that an intronic deletion appears to be associated with a fivefold increase in the expression of WNK1 in affected members and, in this regard, Delaloy et al. (8) have recently shown that sequences between nucleotide positions −2,500 and −1,200 of human WNK1 promoter repress transcription of the gene.

Wilson et al. (45) searched genome and EST databases for WNK1 paralogs, revealing three additional genes encoding WNK isoforms: WNK2 located in human chromosome 9, WNK3 in human chromosome X, and WNK4 in human chromosome 17 within the genetic interval previously linked to PHA-II kindred on this chromosome. Single-strand conforma-
tion polymorphism analysis revealed missense mutations in exon 7 of WNK4 in four different kindred previously linked to this region. In all cases, a charged residue is substituted in a region that is located just beyond the first coiled-coiled domain that contains a cluster of 10 charged residues that are highly conserved in all 4 WNK isoforms (Fig. 1). Therefore, it was proposed that PHA-II in a kindred’s linkage to the chromosome 17p11-q21 region is due to missense mutations of WNK4. The other two genes responsible for PHA-II, one in families linked to chromosome 1q31–42 (23) and another still unmapped (10), remain a mystery, but, interestingly, no WNK or WNK-like gene is located in this region of human chromosome 1, suggesting that completely different proteins are involved.

MOLECULAR BIOLOGY OF THE WNK KINASE FAMILY

WNKs are a group of kinases recently identified by Xu et al. (48) during their search for novel members of the MAP/extracellular signal-regulated protein kinase families. These investigators isolated from a rat brain library a 7.2-kb cDNA encoding a serine/threonine kinase of 2,126 amino acid residues. The human and mouse orthologs were later identified: the first as a 251-kDa protein composed of 2,382 amino acid residues (41) and the second as a 2,377-amino acid protein (30). The degree of identity between human, rat, and mouse WNK1 is 86%. All serine/threonine kinase enzymes that have been identified at the molecular level share a catalytic domain of 300 amino acid residues that is subdivided into 12 domains, with several residues that are strictly conserved. One of these conserved residues is a lysine (K) located in subdomain II that is known to be critical for ATP binding in the catalytic site. Because this new kinase family does not possess the catalytic lysine in subdomain II but exhibit the catalytic lysine on subdomain I (49).

As previously mentioned, three additional members of the family have been identified at the molecular level (Fig. 1): WNK2 composed of 2,216 amino acid residues located in human chromosome 9q22.31 (41), WNK3 composed of 1,800 residues located in human chromosome Xp11.22 (15), and WNK4 composed of 1,243 residues (45) (official nomenclature is PRKWNK2, PRKWNK3, and PRKWNK4, respectively). The overall degree of identity between WNK proteins is ~40%; however, all four kinases exhibit similar topology (Fig. 1). The more conserved region of WNKs is the kinase domain (~79%) located toward the NH2-terminal side of the protein containing the expected 12 subdomains. All four WNKs do not contain the conserved lysine in subdomain II but exhibit the catalytic lysine on subdomain I (49). The other conserved regions are the autoinhibitory domain (49) and two coiled-coil domains. Figure 1 depicts the location of the cluster of conserved charged residues in which mutations in WNK4 have been observed in patients with PHA-II linked to chromosome 17. In addition, there are several PXXP motifs that could potentially interact with SH3 domains of other proteins. Little is known about biochemical properties of WNKs or their substrates. It has been demonstrated that WNK1 contains an autoinhibitory domain between residues 485 and 555 located after the kinase domain but before the first coiled-coil domain (49) (Fig. 1). Two serines located within the activation loop (S382 and S378) appear to regulate kinase activity and have been proposed to be part of the autophosphorylation domain (49). The crystal structure of WNK1 has been recently resolved at 1.8 Å (27). The target pathways of WNK1 are unknown, but evidence has been obtained suggesting that WNK1 is a MAP4K of the ERK5 kinase pathway because it is able to activate ERK5 by a process that can be blocked with kinase-dead mutants MEKK2 or MEKK3 or by the MEK5 inhibitor U-0126 (50). A recent study shows that WNK1 is able to physically interact and phosphorylate several members of the synaptotagmin (Syts) proteins that are known to regulate membrane trafficking and vesicle fusion (21). Two-hybrid screen

![Fig. 1. Lineal representation of structural organization in human WNK (for with no K kinases) proteins. The kinase domain is shown in light shading, the autoinhibitory domain in hatch, and the coiled-coil domain in black. The locations of the negatively charged residues stretch in which WNK4 mutations occur are shown in WNK4. Capital letters depict amino acid residue substitution observed in a patient with pseudohypoaldosteronism type II (PHA-II) linked to chromosome 17 (45).]
analysis showed that WNK1 binds to Syts 1, 2, 3, and 9, but not Syts 4, 7, or 8. In contrast, binding between WNK4 and Syts was not observed.

Tissue distribution reveals that WNKs are widely expressed. By Northern blot analysis, WNK1 has been shown to be predominantly expressed in the kidney, heart, muscle, and testis in humans (41), rats (48), and mice (6). Immunostaining with antibodies directed against WNK1 in mice revealed that in every tissue tested, WNK1 is predominantly located in polarized epithelia. Examples are pancreatic, sweat and hepatic biliary duct, DCT and CD in the kidney, epididymis, colonic crypt, gallbladder, and esophageal epithelia (6). In some epithelia, WNK1 expression is intracytoplasmic, whereas in others it is basolateral. The tissue distribution of WNK2 is not known. By RT-PCR analysis it has been shown that WNK3 is present in all tissues (15). Although it was originally described by Western blot and immunohistochemical analyses that WNK4 exhibits kidney-specific expression, exclusively present in cytoplasm and tight junctions of DCT and CD (45), it was later demonstrated that WNK4 transcript and protein are also present in several epithelial tissues, in which expression is more prominent in tight junctions (17). Interestingly, all epithelia expressing WNK1 and WNK4 are heavily involved in Cl- transport.

The molecular diversity of WNK family is further increased by the existence of several alternative splicing variants that have been described in WNK1 and WNK3. The alternative splicing pattern in WNK1 was simultaneously informed by Delaloy et al. (8) and O’Reilly et al. (30). In both studies, it was observed by Northern blot analysis that there are two WNK1 transcripts of 9.0 and 10.5 kb that are ubiquitously expressed. The difference between these transcripts is the length of the 3’-untranslated region due to the existence of alternative polyadenylation sites (8). In addition, there is a 9.0-kb transcript expressed only in the kidney, which is due to alternative splicing of exons 1–4. Thus this isoform lacks the first 437 amino acid residues that include almost the entire kinase domain (Fig. 1). The transcription of this isoform is under control of an alternative promoter located in intron 4 and is the only WNK1 variant that contains sequences from an extra exon located between exons 4 and 5, which has been designated as exon 4a. Interestingly, within the kidney, this isoform with presumably no kinase activity is predominantly located in DCT. The construct containing intron 4 and exon 4a cloned upstream of the luciferase reporter gene demonstrated intense luciferase activity only when Madin-Darby canine kidney (MDCK) cells were transfected. No reporter activity was observed in Chinese hamster ovary or HEK cells. These observations suggested that an alternative promoter in intron 4 contains strong cell-specific enhancer sequences (8) that were found to be contained within a 157-bp DNA fragment. The comparison of this fragment with promoter regions in other genes that are considered as renal specific revealed similarities of 79 and 83% with TSC and human kallikrein genes, respectively. Because PHA-II clinical features are all explained by distal nephron defects in ion transport processes, together with immunohistochemical evidence that both WNK1 and WNK4 are predominantly expressed in DCT and CD in kidney, prompted investigators to analyze the role of WNKs in distal nephron transport pathways. As consequence, as shown in Fig. 2, the WNK family has emerged as comprising protein kinases with multiple regulatory capacities in distal nephron reabsorption mechanisms (5).

The fact that PHA-II is a mirror image of Gitelman’s disease and that all clinical consequences of WNK1 or WNK4 mutations in PHA-II patients (hypertension, hyperkalemia, metabolic acidosis, hypocalciuria) can be explained by distal nephron defects in ion transport processes, together with immunohistochemical evidence that both WNK1 and WNK4 are predominantly expressed in DCT and CD in kidney, prompted investigators to analyze the role of WNKs in distal nephron ion transport pathways. As consequence, as shown in Fig. 2, the WNK family has emerged as comprising protein kinases with multiple regulatory capacities in distal nephron reabsorption mechanisms (5).

Two independent studies have shown that TSC activity can be regulated by WNK4 and WNK1 (46, 52). Using the Xenopus laevis oocyte expression system, it was observed that TSC activity was reduced by WNK4, because thiazide-sensitive 22Na+ uptake in oocytes was significantly lower when TSC and WNK4 cRNA were coinjected than when TSC cRNA was injected alone. Immunochemistry of oocytes, using anti-TSC polyclonal antibodies (52), or confocal image analysis, using an enhanced green fluorescent protein-TSC construct (46), revealed that reduction of TSC activity was due to a decrease in the amount of TSC present in the plasma membrane. This negative effect of WNK4 on TSC appears to involve phosphorylation of the cotransporter because it was not observed when cRNA from a D318A-mutated WNK4 that lacks kinase activity was used (46). In this regard, preliminary data from our group suggest that PKC-induced TSC phosphorylation reduces the activity of the cotransporter (16, 40). Wilson et al. (46) and Yang et al. (52) also showed that TSC activity and expression were not affected by mouse WNK4 harboring the Q562E (Q565E in human WNK4) mutation observed in a kindred with PHA-II; however, mouse WNK4 with other mutations seen in affected patients, such as E559K and D561A (E562K and D564A in human WNK4), retained the ability to reduce TSC activity (52).

The WNK1 regulation of TSC was also analyzed by Ellison and colleagues (52), who observed that although WNK1 alone had no effect on TSC activity, WNK4-induced inhibition of TSC was prevented by WNK1, suggesting that WNK1 either...
regulates WNK4 kinase activity or counteracts WNK4’s effects on TSC. In this regard, a WNK1-WNK4 physical interaction has been suggested by preliminary evidence (53). Finally, Wilson et al. (46) also presented immunoprecipitation data suggesting that TSC and WNK4 physically interact with each other in a process not affected by WNK4 mutations.

Following these series of observations, both groups proposed that wild-type WNK4 is a natural inhibitor of TSC expression and by preventing this effect of WNK4, PHA-II-type mutations result in an abnormal release of TSC activity (Fig. 2). Thus, as predicted by the mirror image between PHA-II and Gitelman’s syndrome, as well as by the sensitivity to thiazide-type diuretics in PHA-II patients, an increased TSC activity appears to be part of the pathophysiology of this illness in which a chronic increase in salt reabsorption by DCT can potentially produce a salt-dependent form of hypertension in adult life, similar to what occurs in Liddle syndrome (35). Supporting a key role of TSC in blood pressure regulation, Majid and Navar (22) have shown that TSC is the major renal sodium-entry pathway in the distal nephron mediating arterial pressure-induced changes in sodium excretion, and Wang et al. (42) observed that TSC is the only nephron transporter that is downregulated during aldosterone escape, implicating a decrease in TSC expression as an important mechanism for aldosterone-induced high blood pressure to restore natriuresis, despite the continuous presence of a high concentration of aldosterone.

Yang et al. (52) proposed that increased activity and/or expression of WNK1 linked to chromosome 12 (45) observed in PHA-II patients results in pathological inhibition of WNK4, which in turn relieves TSC activity. The deletion of intronic sequences in one allele appears to be enough to increase WNK1 expression (45), thus behaving as a gain-of-function mutation. This proposal for WNK1 fits well with the dominant inheritance pattern of PHA-II and is consistent with observations that blood pressure is significantly lower in mice heterozygous for the WNK1 gene-trap mutation (54). In contrast, WNK4 negative regulation of TSC requires that mutations in WNK4 behave as inactivating types, whereby WNK4 loses its normal activity toward TSC. Although loss of function is most frequently seen in autosomal-recessive diseases, there are clear examples of inherited genetic diseases following an autosomal-dominant pattern, in which affected genes exhibit inactivating mutations, i.e., a loss-of-function mutation in one allele that is
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enough to express the disease. One possible mechanism is haploinsufficiency, when both fully functional alleles are required to sustain normal protein activity. In these cases, a mutation in one allele produces mild disease and a mutation in both alleles produces severe disease. Examples are inactivating mutations of the calcium-sensing receptor producing autosomal-dominant familial benign hypocalciuric hypercalcaemia, when one allele is affected, or autosomal-recessive severe neonatal hyperparathyroidism, when both alleles are mutated (33), or inactivating mutations of the low-density lipoprotein receptor in familial hypercholesterolemia, in which autosomal-dominant disease patients are at a higher risk for developing atherosclerosis at a younger age than the general population, whereas autosomal-recessive patients develop fatal atherosclerosis during childhood (34). The other mechanism is the dominant-negative effect that occurs when products from both alleles interact to be functional. In this case, a loss-of-function mutation in one allele inactivates or reduces activity of the remaining allele. Examples are some types of porphyrias and type I osteogenesis imperfecta (4). The failure to obtain kinase activity by WNK4 in transfected cell preparations has precluded elucidation of the effect of mutations on WNK4 activity (43).

Two other Cl− transport pathways have been recently shown to be downregulated by WNK4. Kahle et al. (17) used RT-PCR and Western blot analysis to demonstrate that WNK4 is expressed in several epithelial tissues outside the kidney, such as pancreas, biliary duct, epididymis, and colonic crypts, in which the intracellular location occurs in both tight junctions and lateral membranes. Because TSC is not expressed in these epithelia, the effect of WNK4 on other Cl− transport mechanisms was analyzed. It was demonstrated in X. laevis oocytes that activity and surface expression of the basolateral isoform of the Na+/K+/2Cl− cotransporter (BSC2/NKCC1) and apical Cl−/HCO3− exchanger CFEX encoded by the SLC26A6 gene are both reduced by coinjection with wild-type WNK4 cRNA. Thus, in addition to TSC, at least two other Cl− influx mechanisms are inhibited by WNK4, suggesting that WNK4 could be a major Cl− influx regulator. Unfortunately, no mutant or kinase-dead WNK4 cRNAs were tested. Thus it is not known whether these effects of WNK4 require kinase activity to be present and whether PHA-II-type mutations abrogate inhibitory effects of WNK4 on the Na+/K+/2Cl− cotransporter and the Cl−/HCO3− exchanger. In this regard, however, it has been shown that WNK4 physically interacts with a recently identified Ste-20-related kinase (PASK or SPAK) (32) that has been suggested to be involved in functional regulation of the basolateral Na+/K+/2Cl− cotransporter (11, 32).

Because one of the prominent features of PHA-II is hyperkalemia, localization of WNK4 in the DCT and CD suggested that a direct effect of WNK4 could occur in the ROMK potassium channel, which is the major K+ secretory pathway in the distal nephron. Supporting this hypothesis, Kahle et al. (19) demonstrated that ROMK activity is significantly reduced by wild-type WNK4 following a mechanism that does not appear to be related to WNK4 kinase activity. A similar inhibitory effect was observed with kinase-dead WNK4 (D318A), which is known to loss the kinase activity, or with a truncated WNK4 construct lacking the first three kinase subdomains. The inhibitory effect of WNK4 on ROMK is due to a clathrin-dependent WNK4-induced reduction of ROMK expression in cell surface and potentially requires ROMK and WNK4 physical interaction because both proteins were shown to form complexes in immunoprecipitation experiments. Thus WNK4 inhibits TSC and ROMK by different mechanisms. TSC inhibition appears to be related to WNK4 kinase activity, whereas this is not required for ROMK reduction, which is due to a clathrin-dependent reduction of ROMK in plasma membrane (Fig. 2).

The fact that WNK4 has been localized to tight junctions in the DCT and CD in the kidney and other epithelial cells (19, 45), together with the so-called chloride shunt hypothesis coined after observations in one patient that sodium sulfate, but not sodium chloride, infusion corrected urinary potassium excretion (36), prompted Yamauchi et al. (51) and Kahle et al. (18) to study the possibility that WNK4 regulates paracellular Cl− transport pathways using transfected MDCK and MDCK II tet-off cells, respectively. Yamauchi et al. (51) were not able to show that wild-type WNK4 had significant effect in paracellular Na+ or Cl− transport but Kahle et al. showed that wild-type WNK4 induced a significant decrease in transepithelial resistance that was secondary to a twofold increase in absolute paracellular chloride permeability. This effect of WNK4 was not observed with the D318A WNK4 mutant that lacks kinase activity. Yamauchi et al. (51) observed that the PHA-II-type mutant WNK4 D564A (D561A in murine WNK4) decreased Na+ and increased Cl− transport by paracellular mechanisms. In addition, it was also observed that wild-type WNK4 induced phosphorylation of claudins 1–4 and that mutant WNK4 D564A further increased phosphorylation of these tight junction proteins. Similarly, Kahle et al. (18) demonstrated that PHA-II-type mutants WNK4 Q562E and E559E increased paracellular Cl− permeability >4.5 times by altering the properties of the preformed tight junctions. These observations suggest that WNK4 may indeed regulate paracellular Cl− transport and that mutations in this respect behave as gain-of-function mutations.

In contrast to what was observed in the effects of wild-type and mutant WNK4 on TSC activity, mouse WNK4 harboring PHA-II-type mutations Q562E and E559K increased the inhibition of K+ current and surface expression induced by the EGFP-ROMK construct and increased paracellular Cl− transport. In addition, human WNK4 with the D564A mutation, which also increased paracellular Cl− transport, increased claudin phosphorylation. Therefore, as shown in Fig. 2, the effects of mutations appear to be opposing. On one hand, reduction of TSC activity is prevented by WNK4 mutations, while on the other hand reduction of ROMK activity and claudin phosphorylation by wild-type WNK4 is further increased by WNK4 mutants. Although there is no study to show that WNK4 mutation D564A, which increases phosphorylation of claudins, also loses the ability to inhibit TSC, these observations suggest the intriguing possibility that mutations in WNK4 behave as a loss of function for TSC but as a gain of function when it comes to ROMK and claudins. Because mutations are present in a cluster of charged residues outside of the kinase domain, it is conceivable that a change in WNK4 net charge or tertiary structure simultaneously affects its kinase activity and the interaction with other proteins. This results in the loss of its inhibitory effect on TSC, probably due to a reduction of its kinase activity toward the cotransporter (kinase-dead effect), and increased interaction with ROMK and
claudins, enhancing its inhibitory effect and phosphorylation of the channel or claudins, respectively. That a single-point mutation induces gain of function and loss of function simultaneously is possible when mutations occur in regulatory proteins such as kinases, because, as a result of a particular mutation, interaction or phosphorylation can be increased for some and decreased for other target proteins. One example of such behavior is the dual phenotypic Janus C620R mutation in the ret gene encoding a transmembrane tyrosine kinase receptor. It is known that gain-of-function mutations in this membrane protein produce different syndromes of multiple endocrine neoplasia type 2A and 2B (MEN), whereas loss-of-function mutations result in a form of inherited colonic aganglionosis syndrome known as Hirschsprung’s disease (HSCR). However, patients with missense mutations in particular residues of ret protein, such as 609, 611, 618, and 620, cosegregate with both MEN and HSCR, suggesting that gain-of-function and loss-of-function mutations coexist. This hypothesis was recently shown to be true by Arihi et al. (2), who observed biochemical and biological evidence that the C620R mutation induced the gain-of-function and loss-of-function effects in ret protein that are known to be associated with NEM and HSCR, respectively.

Dual activating and inactivating mechanisms of WNK4 mutations will also resolve, at least in part, two poorly understood issues in PHA-II. The first is that thiazide sensitivity and the chloride shunt hypothesis will be both possible if WNK4 mutations simultaneously relieve TSC activity and increase paracellular Cl⁻ transport rate. The second is the consistently different period of time required for each of the clinical consequences to be evident. Hyperkalemia, which usually occurs during childhood, could be due to an activating behavior of WNK4 mutations in one affected allele is enough. In contrast, hypertension, which usually does not appear until the third or fourth decade of life, could be the result of inactivating behavior of WNK4 mutations in just one allele, which probably takes longer to express through haploinsufficiency or dominant-negative effects.

Finally, as shown in Fig. 2, the dual effect of WNK4 on the Na⁺-Cl⁻ cotransporter and K⁺ channels is ideal to explain the regulation of salt reabsorption and potassium secretion during physiological challenges such as hypovolemia or hyperkalemia. During hypovolemia, it is possible that regulation of WNK4 relieves TSC but reduces ROMK activity, increasing salt reabsorption without losing K⁺. In contrast, during hyperkalemia, WNK4 regulation inhibits TSC, while ROMK is relieved, maximizing K⁺ secretion, without increasing salt reabsorption. There is still a long way to go to fully understand the function of WNK4 (and WNK1) in the kidney. Regulatory effects of WNK1 or WNK4 in potentially key transport systems such as the epithelial sodium channel, calcium channels (TRPV5 or 6), and K⁺-H⁺-ATPase have not been informed, but it is evident that recent findings have pointed to these kinases as multiregulators of ion transport processes. To date, evidence shows that WNK4 regulates activity of TSC, ROMK, paracellular Cl⁻ pathways, the basolateral Na⁺-K⁺-2Cl⁻ co-transporter, and the Cl⁻/HCO₃⁻ exchanger.

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