Mechanisms of Type I and Type II Pseudohypoaldosteronism

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Nephrologists frequently encounter disorders involving aldosterone. Primary and secondary hyperaldosteronism, conditions in which aldosterone is elevated, are typically characterized by variable levels of volume overload and hypokalemia. Conversely, patients with hypoaldosteronism from primary adrenal insufficiency or hyporeninemic hypoaldosteronism from diabetes frequently display hyperkalemia but may have no signs of volume depletion.

The major actions of aldosterone are well described in the cortical collecting duct. Aldosterone increases the activity of basolateral Na/K ATPase, luminal expression of epithelial sodium channel (ENaC), and the activity of luminal renal outer medullary potassium (ROMK) channels. There is, however, uncertainty regarding the molecular intermediates that transduce these actions. Furthermore, it is unclear why disorders involving changes in levels of aldosterone have different phenotypes. For example, although both hyperkalemia and volume depletion stimulate aldosterone secretion, the actions of aldosterone seem tailored to the stimulus: potassium secretion without volume overload in the first situation and sodium reabsorption without hypokalemia in the latter condition.

Here we briefly review the literature regarding the pathogenesis of both pseudohypoaldosteronism type I (PHAI) and pseudohypoaldosteronism type II (PHAII or Gordon’s syndrome). Although both conditions have been recognized for decades, emerging in vitro and in vivo data provide new insights regarding the biologic effects of aldosterone and the variety of targets through which aldosterone may exert its actions.

PHAI and PHAII

PHAI was first described in 1958 in an infant with recurrent episodes of volume depletion and hyperkalemia.1 More than 100 cases with a similar syndrome (variable levels of salt wasting, metabolic acidosis, and hyperkalemia) have since been described with both autosomal dominant and recessive patterns of inheritance. In 1996, Chang et al.2 reported that autosomal recessive PHAI was caused by mutations of ENaC. Most cases of autosomal dominant PHAI link to mutations in the mineralocorticoid receptor (MR).3 In general, patients with autosomal recessive PHAI, as opposed to patients with autosomal dominant PHAI, have a more severe clinical phenotype and do not spontaneously improve during early childhood. Furthermore, given the generalized expression of ENaC in epithelial tissues, autosomal recessive PHAI associates with systemic manifestations, most notably, recurrent pulmonary infections.

Although most patients with PHAI have an identifiable mutation, there are still significant unknowns regarding the pathogenesis of PHAI. Whereas it is easy to explain that loss of two copies of ENaC will lead to sodium wasting and hyperkalemia, it has been less obvious how losing only one copy of the MR causes disease. Detailed studies of different MR mutations show that mutations can affect the

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N-terminal region, ligand-binding domain, or DNA-binding domain. Although functional studies using various MR reporter assays showed that causative mutations associate with less MR activation in response to aldosterone, Geller et al. studied MR mutations in six families with autosomal dominant PHAI and reported novel insights regarding some mutations. In one kindred, there was a C1984T mutation that converted G590 into a stop codon. In peripheral blood leukocytes, there was only mRNA for wild-type MR and no mRNA for mutant MR. Therefore, rather than acting as a “dominant negative,” PHAI mutations in MR decrease amounts of cellular MR. In this same report, these investigators studied children and adults with PHAI mutations and found adults were phenotypically normal (other than elevated serum aldosterone concentration) and many family members with PHAI mutations never had any episodes of salt wasting or hyperkalemia. Conversely, there were four young children who were at risk for PHAI mutations who died at very young ages, implying PHAI can sometimes present with a more severe phenotype, leading to neonatal mortality. Such data suggest two possibilities for understanding: one is that different MR mutations have different effects on MR activation, and the other is that compensatory pathways modify disease phenotype.

In 1964, the first case of PHAII was reported. Rather than episodes of volume depletion, the teenager patient had hypertension, severe hyperkalemia, and mild metabolic acidosis. Six years later, Gordon et al. reported another case of PHAII. Similar to the first patient, this new patient also had low levels of plasma renin, hyporesponsiveness to exogenous mineralocorticoids, and a good response to thiazide diuretics. Subsequent studies showed that PHAII is transmitted in an autosomal dominant fashion. A major advance in understanding PHAII occurred with the identification of causative mutations. Wilson et al. identified mutations in the WNK1 and WNK4 genes in some patients with PHAII. WNK1 had only been recently described, and the role of WNKs was not yet understood. WNK kinases are so named because of the lack of lysine in the ATP-binding cassette of the catalytic region. Interestingly, patients with WNK1 mutations have intronic mutations that increase the abundance of WNK1, and patients with WNK4 mutations have missense mutations that localize to a short region in a highly conserved WNK residue.

**ROLE OF WNK1 AND WNK4 IN ELECTROLYTE HANDLING**

Because patients with PHAII often have a dramatic response to thiazide diuretics, investigators first focused on the relationship between WNK1, WNK4, and the thiazide-sensitive NCC cotransporter. Indeed, two separate groups identified a relationship between WNK4 and NCC activity using Xenopus oocytes; both groups showed that wild-type WNK4 inhibited surface expression of NCC and, consequently, NCC activity. Mutant WNK4 isoforms have a variable effect on NCC activity, but one WNK4 mutation seen in PHAII, Q562E, seems to diminish the ability of WNK4 to regulate NCC. Given the variable effects of different WNK4 isoforms, identifying precise molecular events that affect NCC activity, or accompanying PHAII mutations in WNK4, is still an active area of research.

To study the mechanism of PHAII-induced hyperkalemia, investigators have focused on links between WNK4 and ROMK potassium channels and chloride transport. Using Xenopus oocytes or mammalian cell lines, wild-type WNK4 decreases surface expression of ROMK, and PHAII-mutant WNK4 decreases it further. Because patients with PHAII have increased urinary potassium excretion after treatment with a non-resorbable anion (such as NaHCO₃) but not NaCl, it has been hypothesized that patients with PHAII have a chloride shunt. In other words, patients have increased distal chloride reabsorption, leading to less electronegativity of the lumen, thereby decreasing the electrochemical gradient for potassium secretion. There is now good in vitro evidence in MDCK cells that wild-type WNK4 increases chloride permeability and PHAII-mutant WNK4 increases chloride permeability even further.

To date, two *in vivo* models have been developed to study the role of WNK4 in mice. Lalioti et al. first described the generation of a WNK4 transgenic mouse. Three mouse populations, wild-type mice, mice expressing one extra copy of wild-type WNK4, and mice expressing two normal copies of WNK4 and one WNK4 with the Q562E mutation, were studied. Mice overexpressing the PHAII mutation in WNK4 display hypertension and hyperkalemia. Additionally, PHAII-mutant WNK4 transgenic mice exhibit metabolic acidosis and hypercalciuria, and the entire phenotype is reversed when the mice are crossed with NCC knockout mice, implying NCC hyperactivity as a major component of the PHAII phenotype.

Subsequently, Yang et al. generated a knock-in model of PHAII. To more closely model human disease, these investigators generated mice with one normal copy of WNK4 and one mutant WNK4 (D561A). Similar to human PHAII, the mice exhibited hypertension and hyperkalemia that improves with thiazide treatment. Urine potassium excretion also increases with Na₂SO₄. Verifying *in vitro* data linking the Q562E mutation in WNK4 to increased NCC activity, WNK4 knock-in mice with a PHAII mutation show increased phosphorylation and surface expression of NCC. Similar to the early report, there is no increase in overall levels of ROMK despite hyperkalemia.

Although there are not yet *in vivo* studies exploring the relationship between WNK1 and electrolyte transporters, there are *in vitro* data showing such a relationship. Because WNK1 mutations producing PHAII lead to increased WNK1 levels, one would predict that WNK1 increases sodium reabsorption and decreases potassium secretion. Indeed, in *Xenopus* oocytes, WNK1 activates NCC activity by reversing the inhibitory effect of WNK4 and increasing cell surface expression of NCC.

In HEK cells, full-length WNK1 inhibits ROMK activity by stimulating endocytosis of ROMK.
DNK ISOFORMS AS DOWNSTREAM TARGETS OF ALDOSTERONE

Because WNK1 and WNK4 mediate biologic effects that associate with aldosterone, there has been interest in linking WNK isoforms with aldosterone action. Based on studies using immunofluorescence, WNK1 and WNK4 localize to the aldosterone-sensitive portions of the tubule (distal convoluted tubule, connecting tubule, and cortical collecting duct). Serum and glucocorticoid-regulated kinase 1 (SGK1) also represents one established link between aldosterone and WNK4. SGK1 transcription is induced by aldosterone and activates ENaC and ROMK by phosphorylation of effector molecules such as Nedd 4-2, a ubiquitin ligase that marks ENaC for degradation. In vitro studies showed that a serine residue (S1169) in WNK4 is phosphorylated by SGK1. After WNK4 is phosphorylated at that site, WNK4 is less able to inhibit ENaC and ROMK. In addition to ENaC and ROMK, aldosterone also increases the activity of NCC. Phosphorylated WNK4 (at S1169 and another residue) inhibits NCC less well, providing a mechanism for aldosterone-induced activation of NCC.17

Angiotensin II also seems to directly affect WNK4 and NCC activity. A recent study by San-Cristobal et al.18 showed that angiotensin II can relieve the inhibitory effect of WNK4 on NCC. Furthermore, the stimulatory effects of PHAII mutant WNK4 on NCC cannot be increased further by angiotensin II. The effects of angiotensin II are mediated by Ste20-related serine-threonine proline-alanine rich kinase, a kinase known to affect NCC.

There also is additional evidence that the effects of aldosterone may be mediated through WNK1. There are two transcript variants of WNK1: a full-length WNK1 with a kinase domain that is located in many tissues (L-WNK1) and a short transcript without a kinase domain located exclusively in the kidney (ks-WNK1). Global WNK1 heterozygotes show a phenotype consistent with hyperkalemia. As more molecular targets of WNK1 and WNK4 are identified in the distal nephron, aldosterone signaling in the distal nephron will also likely come of age.

DISCLOSURES

None.

REFERENCES


Figure 1. Proposed relationship between WNK4 and mediators of the RAAS. In absence of aldosterone or angiotensin II (left), WNK4 exerts a tonic inhibitory effect on mediators of Na reabsorption (NCC, ENaC, Cl reabsorption), as well as potassium secretion (ROMK). (Right) A proposed system in which aldosterone and angiotensin II can affect WNK4 activity. In the absence of angiotensin II (hyperkalemia), SGK1 phosphorylates and inactivates WNK4, resulting in abrogation of WNK4 inhibition of NCC, ENaC, Cl channels, and ROMK. In the presence of the PHAII mutation, WNK4 is presumably phosphorylated and is no longer able to inhibit Na and Cl transporters, but inhibition of ROMK is augmented, leading to hyperkalemia. In high angiotensin II states (such as volume depletion), WNK4 inhibition of NCC is reversed through a (WNK4-dependent) SPAK pathway, whereas WNK4 inhibition of ROMK is abrogated through SGK1 phosphorylation and inactivation of WNK4. Ks, K secretion; Na, Na reabsorption; NCC, NaCl cotransporter; ENaC, epithelial Na channel; ROMK, renal outer medullary K channel; SGK1, serum and glucocorticoid-induced kinase 1; SPAK, Ste20-related serine threonine proline alanine-rich kinase.