Renal Tubular Sodium Transporters and Genetic Basis of Hypertension

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Abstract

Renal regulation of total body sodium (Na+) plays an important role in volume-dependent blood pressure control through different molecular mechanisms. Gain of renal tubular Na+ transporter function in different segments is primarily responsible for essential and monogenic forms of hypertension. In this review, we summarize the physiological regulation of the major renal tubular Na+ transporters and recent findings of molecular pathogenesis regarding the genetic basis of hypertension with an activated or enhanced expression of Na+-hydrogen exchanger isoform 3 (NHE3), Na+/potassium (K+) ATPase, renal-specific Na+-K+-2chloride (Cl-) cotransporter isoform 2 (NKCC2), Na+-Cl- cotransporter (NCC) and epithelial Na+ channel (ENaC) in the renal tubules.

KEY WORDS: hypertension, transporter, signaling

Introduction

The kidney plays an essential role in blood pressure regulation by controlling sodium (Na+) homeostasis. In normal adult kidneys, around 25,000 mEq (600 gram) Na+ is filtrated into the renal tubules daily and most of the filtrated Na+ (99.9-99%) is re-absorbed by the renal tubules, resulting in only 0.1-1% of the filtrated Na+ excreted in the urine (1). In addition to impaired Na+ excretion in chronic kidney disease, augmented renal Na+ reabsorption with an intact glomerular filtration rate may also lead to hypertension (2).

Although hypertension is a multifactorial disease probably resulting from the multiple environmental determinants, metabolic disorders and inheritance of a number of susceptibility genes, growing evidence from animal models, human twin and family studies suggests that genetic contribution to blood pressure variation is about 30-60% (3). With the advantage of advances in gene study technology, several hypertension-related candidate gene polymorphisms or mutations on the renal tubule Na+ transporters and their upstream regulators had been reported (4). In polygenic human essential hypertension, the major increase in Na+ transport occurs in the proximal tubule (PT) and thick ascending limbs (TAL) of loop of Henle’s (LOH) (Table 1) (5, 6). On the other hand, distal renal tubules [including distal convoluted tubule (DCT) and downstream distal collecting ducts (CD)] play a dominant role in mo-

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Table 1. Role of major proximal tubules and thick ascending limbs Na+ transporters in human and rodent polygenic hypertension

<table>
<thead>
<tr>
<th></th>
<th>Human</th>
<th>Rodent</th>
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<tr>
<td>NHE activity†</td>
<td>activity†/protein†</td>
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<tr>
<td>Na+/K+ ATPase activity†</td>
<td>activity†/protein†</td>
<td></td>
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<tr>
<td>NBC1 mRNA†</td>
<td>NA</td>
<td></td>
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<tr>
<td>NKCC1 activity†</td>
<td>activity†</td>
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<tr>
<td>NKCC2 NA</td>
<td>surface to intracellular ratio†</td>
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NA: not analysis
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<thead>
<tr>
<th>Syndrome</th>
<th>Gene</th>
<th>Pattern</th>
<th>PRA/PAC</th>
<th>Acid-Base</th>
<th>Age of Onset</th>
<th>Mechanism</th>
<th>Treatment</th>
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<tr>
<td><strong>Distal Tubule</strong></td>
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<tr>
<td>1. Pseudohypoaldosteronism type II (PHA II)</td>
<td>WNK1(12p)</td>
<td>AD</td>
<td>↑/↓↑</td>
<td>↑/Acidosis</td>
<td>Child/Adult</td>
<td>1. Activation WNK1/4-SPAK/OSR1-NCC phosphorylation signaling with increased NCC apical sorting. 2. Reducing NCC-KLHL3/Cul3 complex with less NCC ubiquitination and endocytosis.</td>
<td>Thiazide</td>
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<tr>
<td></td>
<td>WNK4(17q)</td>
<td>AD</td>
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<td></td>
<td>KLHL3(5p)</td>
<td>AD/AR</td>
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<td></td>
<td>Cul3(2q)</td>
<td>AD</td>
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<td>2. Liddle’s syndrome (LS)</td>
<td>ENaCβ(12p)</td>
<td>AD</td>
<td>↓↓↓</td>
<td>↓/Alkalosis</td>
<td>Child/Adult</td>
<td>• PPXY motif mutation in C-terminus contributing to impaired ENaC endocytosis</td>
<td>ENaC Inhibitors</td>
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<td></td>
<td>ENaCγ(16P)</td>
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<td>3. Hypertension exacerbated by pregnancy (H-P)</td>
<td>NR3C2(4q)</td>
<td>AD</td>
<td>↓/≈</td>
<td>↓/Alkalosis</td>
<td>Child/Adult</td>
<td>• Activating MR not only by aldosterone but also by ligands that are normally silent or antagonistic (<em>e.g.</em> progesterone)</td>
<td>ENaC Inhibitors</td>
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<tr>
<td>4. Apparent mineralocorticoid excess (AME)</td>
<td>11βHSD2(16q)</td>
<td>AR</td>
<td>↓/↓</td>
<td>↓/Alkalosis</td>
<td>Infant/Child/Adult</td>
<td>• Impaired conversion of cortisol to cortisone Resulting in activation of MR by cortisol in PC.</td>
<td>MR Inhibitors</td>
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<td><strong>Adrenal Gland</strong></td>
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<td>5. Familial hyperaldosteronism type I (FH I; GRA)</td>
<td>CYP11β1/2(8q)</td>
<td>AR</td>
<td>↑/↓</td>
<td>↓/Alkalosis</td>
<td>Infant/Child</td>
<td>• Chimeric gene due to gene duplication and unequal crossing over between aldosterone synthase (<em>CYP11β2</em>) and steroid 11β-hydroxylase (<em>CYP11β1</em>) resulting in regulation of aldosterone synthase by ACTH.</td>
<td>Dexamethasone MR Inhibitors</td>
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<tr>
<td>6. Familial hyperaldosteronism type II (FH II)</td>
<td>Unknown(7p)</td>
<td>AD</td>
<td>↑/↓</td>
<td>↓/Alkalosis</td>
<td>Adult</td>
<td>• Increasing mineracorticoid production.</td>
<td>MR Inhibitors</td>
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<tr>
<td>7. Familial hyperaldosteronism type III (FH III)</td>
<td>KCNJ5(11q)</td>
<td>AD</td>
<td>↑/↓</td>
<td>↓/Alkalosis</td>
<td>Adult</td>
<td>• Mutant KCNJ5 channels conduct Na⁺, resulting Na⁺ entry, membrane depolarization and aldosterone production and cell proliferation.</td>
<td>MR Inhibitors</td>
</tr>
<tr>
<td>8. Congenital adrenal hyperplasia (CAH)</td>
<td>CYP11β1(8q)</td>
<td>AR</td>
<td>↓/↓</td>
<td>↓/Alkalosis</td>
<td>Infant</td>
<td>• Impaired normal synthesis of cortisol resulting in increasing the production of deoxycorticosterone (DOC), a precursor of aldosterone with mineracorticoid effect.</td>
<td>Dexamethasone ENaC inhibitors</td>
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<td>CYP17α1(10q)</td>
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<td>9. Familial glucocorticoid resistance (FGR)</td>
<td>NR3C1(5q)</td>
<td>AR/AD</td>
<td>↓/≈↑</td>
<td>↓/Alkalosis</td>
<td>Infant</td>
<td>• Glucocorticoid receptor resistance to feedback of cortisol concentration with the consequence of cortisol and androgen overproduction.</td>
<td>Dexamethasone MR Inhibitors</td>
</tr>
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</table>

nogenic (Mendelian) hypertension, which accounts for less than 1% of human hypertension (Table 2) (7). In this review, we discuss the physiological regulation and genetic molecular pathogenesis regarding the renal tubule Na\(^+\) transporters involved in both essential and monogenic forms of hypertension.

**Proximal Tubular Na\(^+\) Transporter**

**Apical Membrane**

The major Na\(^+\) transporters include sodium-hydrogen exchanger isoform 3 (NHE3, *SLC9A3*), sodium phosphate cotransporter type 2 (NaPi2, *SLC34A3*), sodium glucose transporter (SGLT), and sodium amino acid transporters (Fig. 1). Among these transporters, NHE3 plays a main role in Na\(^+\) reabsorption in the PT and is crucial for blood pressure regulation. Physiologically, angiotensin II, aldosterone and norepinephrine, insulin, thyroid hormone, glucocorticoid, androgen, acute acid loading and sympathetic nervous system can enhance renal Na\(^+\) reabsorption partially through increase in NHE3 expression and activity, while endothelin-1, glucagon, dopamine, nitric oxide (NO), parathyroid hormone (PTH) and chronic acidosis all exert an inhibitory effect (8, 9).

In renal NHE3-deficient mice, lower blood pressure was observed (10). Although none of the mutations or single nucleotide polymorphisms (SNPs) in the NHE3 (*SLC9A3*) gene have been reported to be associated with essential hypertension (11), genetic and acquired hypertension is related to increased NHE3 activity in spontaneous hypertension rat (SHR) (12, 13). An analysis of NHE activity in the erythrocytes from normotensive and hypertensive patients also showed that around half of the essential hypertensive subjects reveal an increased NHE activity (14).

**Basolateral Membrane**

Sodium-potassium-adenosine triphosphatase (Na\(^+/K\(^+\) ATPase, primary active) and sodium bicarbonate cotransporter type 1 (NBC1, *SLC4A4*) are responsible for the major role in Na\(^+\) reclaim. Increased Na\(^+/K\(^+\) ATPase activity has been reported to be associated with essential hypertension. Dopamine and ouabain, an ACTH-stimulated endogenous cardiolid secreted dominantly from adrenal gland, both inhibit Na\(^+/K\(^+\) ATPase activity (15, 16). Chronic administration of exogenous ouabain induces hypertension in rat and elevated plasma ouabain is directly correlated with increased blood pressure in essential hypertension (17). In Milan hypertensive rats, antagonizing the pressure effect of ouabain by rostafuroxin can normalize the ouabain-dependent up-regulation of the renal Na\(^+/K\(^+\) ATPase, thus reducing blood pressure (18).

NBC1 is responsible for the majority (~80%) of reabsorption of filtered HCO\(_3^-\) in the proximal tubules. Norepinephrine, angiotensin II, dopamine, glucocorticoid and NO serve as strong physiological stimulators of NBC1. *SLC4A4* gene has recently been found to be one of the hypertension susceptibility genes with a higher expression in Han Chinese hypertensive patients (19).

**Thick Ascending Limb Na\(^+\) Transporter**

**Apical Membrane**

Renal-specific Na\(^+\)-K\(^+\)-2chloride (Cl\(^-\)) cotransporter isoform 2 (NKCC2) is responsible for decreasing gradually TAL luminal NaCl concentration from 140 mM in the inner stripe of the outer medulla to 30-60 mM at the macula densa (20). As shown in *in vitro* studies, NKCC2 can be phosphorylated and activated by the PKA (protein kinase A) and SPAK [STE20 (sterile 20)/SPS1-related proline/alanine-rich kinase]/OSR1 (Oxidative Stress-Response kinase 1) kinases – the downstream substrates of WNK (With-
no-lysine (K) kinases 1 and 4 (21, 22). Physiologically, vasopressin, PTH, glucagon and norepinephrine (through β-adrenergic receptor) can activate NKCC2 through PKA by increasing intracellular cAMP levels (Fig. 2) (23). Osmotic or low intracellular chloride stress had been known to stimulate NKCC2 through WNK1-OSR1/SPAK signaling by *in vitro* research (24). However, little is known about the physiological mechanisms that stimulate SPAK/OSR1 *in vivo*.

Whether norepinephrine, vasopressin or other hormones could also activate NKCC2 through WNK-OSR1/SPAK pathway merits further verification. Although loss-of-function mutation in *SLC12A1* gene-encoding NKCC2 has been well known as the leading cause of Bartter syndrome (BS) characterized by renal salt-losing hypotension with metabolic alkalosis and hypokalemia (25), none of the gain-of-function mutation in NKCC2 gene-causing monogenic hypertension in human has been reported. Similar to the increased NHE activity in the erythrocytes of SHR and essential hypertensive subjects, the activity of the ubiquitously expressed NKCC1 (also found in the basolateral aspect of TAL to downstream tubules) is also increased in the erythrocytes of 20-25% of essential hypertension patients with low plasma renin activity (14, 26). In SHR, increased NKCC2 surface-to-intracellular ratio was observed in accordance with the transition from prehypertensive to hypertensive age (12, 27).

**Basolateral Membrane**

Two isoform of kidney-specific chloride channels (ClC-Ka and b) are responsible for the major role in Cl⁻ reclaim. ClC-Kb is expressed from the TAL to downstream tubules, thus loss-of-function mutation can cause both Bartter syndrome and Gitelman syndrome (28). ClC-Kb T481S gain-of-function mutation by 7- to 20-fold increase in activity was proved to have mildly increased blood pressure (by 5-10 mmHg) and the prevalence of hypertension was twice as high in ClC-Kb S481 carriers than ClC-Kb T481 individuals in Caucasian and African populations (29).

**Distal Convoluted Tubular Na⁺ Transporter**

For the apically expressed Na⁺-Cl⁻ cotransporter (NCC) in the DCT, loss-of-function mutation in *SLC12A3* gene-encoding NCC has been well known as the leading cause of Gitelman syndrome (GS) (30). SNPs in *SLC12A3* gene (Arg904Gln, Thr465Thr, Gly264Ala, C1420T, C1784T and G2736A) have been found to be correlated with essential hypertension (31). Moreover, a kind of monogenic hypertension in human known as pseudohypoaldosteronism type II (PHAII, also called Gordon syndrome or familiar hyperkalemic hypertension), showing the mirror image of GS with salt-sensitive hypertension, hyperkalemia and metabolic acidosis, is sensitive and responsive to thiazide diuretic (a NCC inhibitor) treatment, suggesting that gain-of-NCC function is involved in the fundamental pathophysiology of PHAII (32). Now, it is known that mutations in *WNK1* and 4 genes are responsible for ~10% of PHAII kindreds (33) and the remaining affected kindreds are associated with the newly reported mutations in Kelch-like 3 (KLHL3) (~40%) or cullin 3 (~30%) (34). The exact mechanisms that mutations in KLHL3 and cullin 3 lead to PHAII remain unclear and are probably related to impaired NCC ubiquitination and endocytosis (Table 2). In addition to NKCC1/2, WNK1/4 can also phosphorylate and activate NCC through SPAK/OSR1. An enhanced SPAK/OSR1-NCC phosphorylation signaling was observed in kidney tissues of PHAII-WNK4 mutant knock-in mice, proving the notion that activation of WNK4-SPAK/OSR1-NCC phosphorylation signal cascade serves as the major pathogenesis of PHAII with WNK4 mutation (31, 35).

Clinically, several studies had revealed that mutations or SNPs in WNK1, WNK4, SPAK genes are correlated with essential hypertension, suggesting that activation of WNK1/4-SPAK/OSR1-N(K)CC signaling plays a crucial role in TAL and DCT salt regulation in essential hypertension (31). Recent *in vitro* and *in vivo* studies also showed that WNK-SPAK/OSR1 signaling cascade was involved in the phosphorylation and activation of NCC by norepinephrine (through β-adrenergic receptor), angiotensin II, vasopressin, aldosterone as well as insulin (Fig. 3) (36-38). These findings also suggested that WNK-
SPAK/OSR1 signaling may play a very crucial role in metabolic syndrome-associated hypertension.

Since OSR1 and SPAK share high homology in their catalytic and regulatory domains and their expression in tissues often overlap, we teased apart the role of each kinase in vivo by generating SPAK and OSR1 knockout mice, respectively, and found that SPAK and OSR1 knockout mice manifested hypotension as well as Gitelman- and Bartter-like syndrome, respectively, confirming that OSR1/SPAK are the dominant upstream regulators of NKCC2/NCC (Figs. 2 and 3, respectively) (31, 39).

In addition to NCC-KLHL3-CUL3 complexes, Ras guanyl-releasing protein (RasGRP1) could also increase NCC ubiquitination and endocytosis through activating extracellular signal-regulated kinase (ERK) 1/2 mitogen-activated protein kinase (MAPK) (40). PTH had been identified to induce suppression of NCC activity via stimulation of RasGRP1-ERK1/2 MAPK pathway (Fig. 3) (41). Recent in vitro research also revealed that NCC could interact with adaptor protein 3 (AP3), a lysosomal sorting-related protein, and knock-down of AP3 expression could increase NCC expression (42). In Han Chinese, the gene expression levels of S1 subunit of AP3 in hypertensive patients are lower than those in normotensive individuals by a value of 0.389 on average (19), suggesting that AP3 function may affect blood pressure through regulating NCC expression.

Collecting Duct Na⁺ Transporter

In the renal tubule downstream to DCT comprising cortical connect tubule (CNT), and cortical and medullary collecting duct (CCD, MCD), NaCl reabsorption is mainly through principal cell (PC)-expressed epithelial Na⁺ channel (ENaC), composed of three homologous α, β and γ subunits as a heteromeric channel. Aldosterone, the most important regulator of ENaC, stimulates ENaC α, β and γ subunits synthesis by binding the mineralocorticoid receptor (MR) in the cytoplasma of PCs (43). In PCs, transcription of serum and glucocorticoid-inducible serine/threonine protein kinase 1 (SGK1) is dramatically increased by mineralocorticoids. SGK1 can enhance the ENaC expression by reducing its ubiquitination through phosphorylation of the ubiquitin ligase Nedd4-2. Polymorphisms in SGK1 gene is also reported to correlate with increased blood pressure (44). In addition to aldosterone, vasopressin and insulin could also activate ENaC expression through SGK1 in PCs (Fig. 4) (45, 46). However, SGK1 knockout mice presented hypotension only during low Na⁺ diet, which suggests that ENaC function is partially, not completely, dependent on SGK1. As shown in in vitro research, both WNK1 and 4 could

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**Fig. 3.** Schema of the major Na⁺ and Cl⁻ in the distal convoluted tubule and its intracellular and physiological upstream regulators. SPAK plays a more dominant role than OSR1 in NCC phosphorylation (p). ① represents the monogenic form of hypertension listed in Table 1. p: phosphorylation.

**Fig. 4.** Schema of the major Na⁺ and Cl⁻ in the collecting duct and its intracellular and physiological upstream regulators. ②-③ represent the monogenic form of hypertension listed in Table 1. 11β-HSD2: 11β-hydroxysteroid dehydrogenase type 2. MR: mineralocorticoid receptor.
also activate ENaC through SGK1, which mimics the WNK1/4 activating NCC through SPAK/OSR1 (47).

The term of “monogenic mineralocorticoid hypertension” (MMH) refers to hypertension caused by a single gene mutation, which leads to activation of ENaC characterized by low renin and aldosterone concentration, salt-sensitive hypertension and hypokalemic metabolic alkalosis. MMH patients are sensitive to ENaC inhibitors, such as amiloride and triamterene (Table 1) (48). Liddle’s syndrome is caused by mutations in the Nedd4-2 ubiquitination ligase binding motif (PPXY) located in the cytosolic C-terminus of β and γ subunits leading to defective endocytosis of the ENaC and thus constitutive retention and activation of ENaC in the apical membrane of PCs (49, 50). A potentially GT dinucleotide short tandem repeat in the intron 8 of β subunit of ENaC gene (SCCN1B) presenting in essential hypertension of Chilean population had recently been reported (51). Furthermore, gain-of-function mutation (S810L) in the MR gene (NR3C2) allows MR to be activated by steroids lacking 21-hydroxyl groups, such as spironolactone (MR inhibitor), and progesterone presenting as the early-onset hypertension exacerbated by pregnancy (H-P) (52). In addition, SNPs in the NR3C2 gene including the G allele of the rs5522 is associated with the risk of essential hypertension in Spanish population (53). On the contrary, human with ENaC or MR loss-of-function mutations known as pseudohypoaldosteronism type I (PHAI) presented with ENaC or MR loss-of-function mutations known as pseudohypoaldosteronism type II (PHAI-II/III, congenital adrenal hyperplasia (CAH), and familial glucocorticoid resistance (FGR)). Please refer to the references (58-61) for the detailed genetic basis of MMH summarized in Table 2.

Conclusion

Correlating the SNP mutations or changes of the major renal tubular Na+ transporters and their upstream regulators with essential or monogenic hypertension contributes to our understanding of the molecular pathophysiological role of kidney on volume-dependent blood pressure regulation. The observation from WNK4, SPAK and OSR1 knockin or knockout mice also suggested that development of the inhibitor for N(\(K\))CC upstream regulators (such as WNKs/SPAK/OSR1) promises new drugs for hypertension control.

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