

The Role of Aldosterone in Renal Sodium Transport

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Aldosterone is the body's major hormone involved in volume homeostasis because of its effects on sodium reabsorption in the distal nephron. Our comprehension of the signaling pathways that this mineralocorticoid unleashes has been enhanced through the convergence of bedside physiologic observations with advances in medical genetics and molecular biology. This overview updates our current understanding of the aldosterone-initiated pathways throughout the distal nephron to promote sodium retention. Three essential features of the pathways are explored: how the mineralocorticoid gains specificity and targets gene transcription in distal tubular cells; how the key endpoints of aldosterone action in these cells—the epithelial sodium channel, the thiazide-sensitive sodium chloride cotransporter, and Na,K,ATPase—are regulated; and how 3 kinases, directly or indirectly, are activated by aldosterone and serve as critical intermediaries in regulating the sodium transporters. Remarkably, perturbations in many genes integral to aldosterone-induced pathways result in blood-pressure abnormalities. The familial disorders of hypertension and hypotension that follow from these mutated genes are presented with their molecular and physiologic consequences. The clustering of so many genetic disorders within the aldosterone-sensitive distal nephron supports the hypothesis that renal sodium regulation plays a pivotal role in long-term blood-pressure control. Identifying and characterizing other components of the pathways that modulate these sodium transporters represent the core challenges in this scientific field. It is posited that meeting these challenges will help elucidate the pathogenesis of human hypertension and provide new therapeutic options for its treatment.

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Aldosterone is the chief mineralocorticoid of the body. In the kidney it is responsible for regulating volume homeostasis through its actions on sodium transport in the distal nephron. It also directly augments potassium secretion and indirectly affects physiologic pH through its actions in the collecting duct.

Since the isolation of aldosterone half a century ago, the characterization of the hormone's action within the kidney has mirrored technical progress in biological sciences. In the 1950s and 1960s, astute bedside observations of patients suffering the consequences of altered regulation of aldosterone or its action on the kidney provided the academic stim-

ulus toward unraveling the hormone's molecular signaling pathways. Several Mendelian disorders, such as Liddle's syndrome or pseudohypoaldosteronism type Ia (PHA1a), were described in patients who presented with combinations of electrolyte, acid/base, or blood-pressure perturbations.^{1,2} In particular, patients presented with severe hypertension or, in other disorders, frequent episodes of hypotension. Subsequent phenotypic characterization of these disorders led to an improved understanding of renal sodium handling by the nephron and toward establishing the distal nephron as the hormone's site of action in the kidney. In the past 15 years, genotype specificity for these disorders has been established.³

Because aldosterone was shown to play a significant role in affecting human blood pressure and other conditions such as congestive heart failure, medications have been developed that interfere with aldosterone's production or action. These medications include angiotensin-converting enzyme inhibitors and angiotensin-receptor blockers, both of which inhibit the hormone's production. Within the kidney, distal nephron diuretics and mineralocorticoid-receptor antagonists established their efficacy in altering this pathway. With

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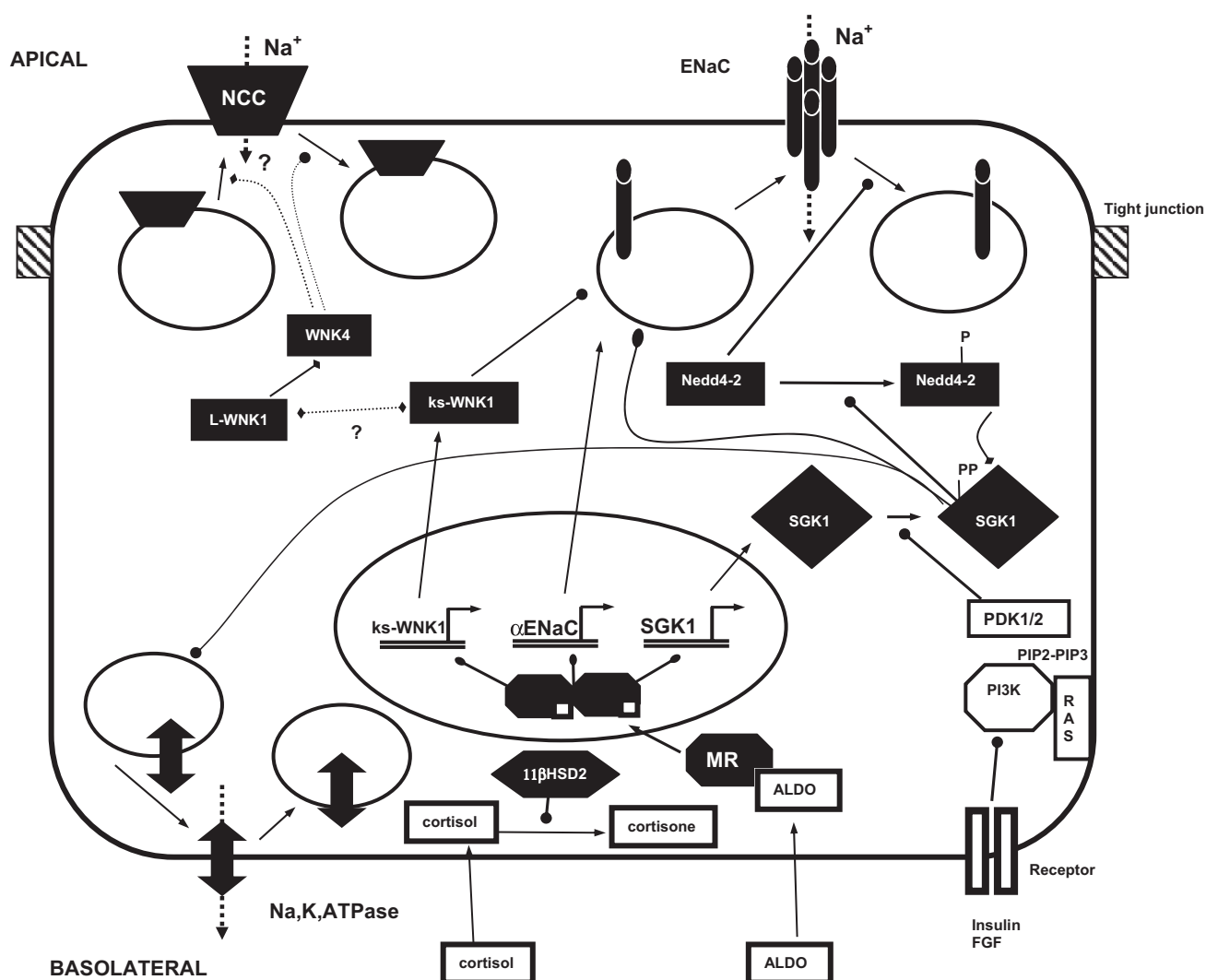


Figure 1 Regulatory mechanisms initiated by aldosterone (ALDO) in DCT2 cells to increase transepithelial sodium transport. Darkened shapes represent key signaling molecules of the pathway that are discussed in greater detail in the text. Pointed arrows represent movement of the molecules within the cell's nucleus, cytoplasm, or subsurface membrane vesicles. Circle-end arrows represent stimulatory mechanisms, diamond-end arrows represent inhibitory mechanisms. Stippled lines with nearby "?" indicate unresolved interactions. Dashed arrows indicate the direction of Na^+ reabsorption from the apical to the basolateral compartments.

the prevalence of hypertension in the general population already high and increasing rapidly among adolescents, these medications and others not yet discovered are expected to play critical roles in preventing morbidity associated with this cardiovascular risk factor.^{4,5}

By concentrating on the functions of aldosterone in supporting and regulating human blood pressure, this article summarizes the molecular actions of aldosterone in handling renal sodium. This article explores both the early and late processes of sodium regulation that the hormone initiates, focusing in particular on the hormonal control of transepithelial sodium reabsorption (see Fig 1). This article does not delve into the mechanisms of aldosterone escape, a renal physiology-mediated adaptive process to prolonged mineralocorticoid exposure.⁶ Although not the focus of the article, the regulation of potassium secretion by aldosterone is summarized in the context of how this process is distinguished

from sodium reabsorption at the molecular level.⁷ This article identifies and discusses the molecules involved in these signaling cascades, and how they may become novel targets for diagnostic and therapeutic interventions against hypertension.

Aldosterone: Synthesis and Physiologic Function

Aldosterone is synthesized and released from the zona glomerulosa cells of the adrenal cortex. Its release is stimulated most strongly by two separate physiologic effectors: hypovolemia and hyperkalemia. Within the nephron, hypovolemia yields a reduced filtrate volume that, after traversing the proximal tubule and loop of Henle segments, delivers a reduced amount of NaCl to the cells of the juxtaglomerular

apparatus. Sensing the lower quantity of NaCl, the juxtaglomerular apparatus cells release renin, which initiates a cascading mechanism that results in angiotensin II production. Angiotensin II directly promotes the secretion of aldosterone, completing the renin-angiotensin-aldosterone axis, and stimulating mechanisms that improve volume homeostasis through retention of sodium in the distal nephron.

Hyperkalemia also stimulates the release of aldosterone from adrenal cortical cells. Although the molecular mechanism of hyperkalemia-induced aldosterone secretion in the adrenal gland has been characterized partially, what is germane for this review is that secreted aldosterone promotes a general kaliuresis, and not necessarily volume retention. How the kidney differentiates between these two distinct aldosterone-mediated responses remains elusive. Its mystery commonly is referred to as the *aldosterone paradox*.⁸

Aldosterone and the Mineralocorticoid Receptor

Aldosterone regulates sodium reabsorption through its actions in the distal nephron. It works chiefly on principal cells of the cortical and medullary collecting ducts. Less appreciated, aldosterone also affects transepithelial sodium transport in cells of the early distal nephron, including the later distal convoluted tubule (DCT2) and the connecting tubules.⁷ Because aldosterone shares agonist properties with other endogenous corticosteroids and glucocorticoids, specificity of its action in epithelial cells requires the presence of 11- β hydroxysteroid dehydrogenase type 2 (11- β -HSD2), an enzyme that catabolizes glucocorticoids into inactive metabolites, preferentially exposing distal tubular cells to mineralocorticoid effects. Indeed, the specificity of mineralocorticoid action in other tissues involved in volume homeostasis, such as the colon, also depends on the expression of 11- β -HSD2.⁹

Aldosterone has been shown to modulate nonepithelial cells in the brain, heart, and vasculature, but 11- β -HSD2 is not required for its action. In these tissues the hormone provokes fibrosis and endothelial dysfunction. Although this subject is not explored further here, their clinical impact was highlighted in the Randomized Aldactone Evaluation Study (RALES) report in which patients with congestive heart failure benefited from mineralocorticoid antagonists.^{10,11}

The primary mechanism that initiates aldosterone's action in principal and other cells of the distal nephron is its interaction with the mineralocorticoid receptor (MR). Also known as the glucocorticoid receptor-2 because it has similar binding affinities for aldosterone and cortisol, MR exists unbound by hormone within the cell's cytoplasm in a complex with several proteins, including heat-shock proteins. Because 11- β -HSD2 catabolizes competing glucocorticoids into inactive products, aldosterone binds MR. This ligand-receptor complex traverses into the cell's nucleus and forms a homodimer; that is, it binds to another aldosterone-MR complex. This homodimer then binds double-stranded DNA at specific locations and induces the transcription of aldosterone-sensitive genes.¹² Several genes are activated by this mechanism to

support Na⁺ reabsorption and they are discussed in detail later. To be sure, some investigations report that aldosterone can induce Na⁺ transport in renal tubular cells through mechanisms independent of binding to MR. The physiologic impact from these studies is less well understood.¹³

Two genetic diseases, apparent mineralocorticoid excess (AME) and PHA1b, show the importance of tissue specificity in governing aldosterone's action in maintaining blood-pressure control. AME is a rare autosomal-recessive disease represented by a mutation of the 11- β -HSD2 gene, yielding an inactive enzyme.¹⁴ With cortisol preserved in distal tubular cells and not inactivated into cortisone, MR is unprotected from the effects of glucocorticoids. The clinical consequences of this mutation include severe sodium-sensitive hypertension, hypokalemia, and metabolic alkalosis, despite low plasma renin activity and low serum aldosterone levels. The clinical presentation also can be acquired from excessive ingestion of black licorice, which contains the 11- β -HSD2 antagonist glycyrrhetic acid. Treatment for AME requires spiro lactone or thiazide diuretics. The efficacy of thiazides is especially interesting because it implicates the thiazide-sensitive sodium chloride cotransporter not only in the pathogenesis of AME, but also as a major effector of mineralocorticoid action.

In comparison to patients with AME, PHA1b patients present with the reverse clinical picture. These patients characteristically have low blood pressure, hyperkalemia, and metabolic acidosis, with plasma renin activity and serum aldosterone levels both increased because of hypovolemia. The disorder is caused by a missense, frameshift, or splice sequence mutation to the mineralocorticoid-receptor gene, resulting in a phenotypic reduction in aldosterone sensitivity.^{15,16} The inheritance pattern is autosomal dominant, with the natural history of disease varying by the mutation. The inheritance pattern suggests that the mechanism for homodimerization of ligand-bound MR plays a critical role in fine-tuning aldosterone action on renal tubular cells. Treatment for PHA1b includes sodium loading to maintain volume and lessen the indirect effects from potassium and acid imbalance. In addition, fludrocortisone (a mineralocorticoid) or carbenoxolone (an antagonist of 11- β -HSD2) can be used as treatment to maximize any potential steroid action. The clinical consequences of PHA1b on other MR-expressing organs is not well understood.

Aldosterone and its Regulation of the Transepithelial Sodium Transporters

As previously discussed, renal tubular cells that express MR and 11- β -HSD2 are sensitive to aldosterone. The cells with these characteristics comprise the distal nephron segments: DCT2, connecting tubule, cortical collecting duct, and medullary collecting duct.⁷ Within these segments of the nephron, aldosterone exerts strong control over transepithelial sodium transport systems and fine-tunes physiologic vol-

ume status. On the apical surface of these polarized tubular cells, the epithelial sodium channel (ENaC) is the best-studied target of aldosterone, but the thiazide-sensitive sodium chloride cotransporter (NCC) also is regulated. On the basolateral surface of these tubular cells, experimental evidence suggests that the Na,K,adenosine triphosphatase (Na,K,AT-Pase) is mediated in part by mineralocorticoids. Although aldosterone acts by regulating gene transcription, these sodium transporters are generally not the immediate targets of the hormone. Rather, aldosterone influences the transcription of genes that encode for intermediary proteins within the pathways that control these sodium transporter activities.

Each sodium transporter is reviewed separately before considering how the aldosterone-sensitive genes regulate it. ENaC comprises the bulk of the discussion because empirically it has been the most studied and its function is controlled tightly by aldosterone.

ENaC

The epithelial sodium channel comprises three subunits (α , β , and γ) that likely form a tetramer.¹⁷ Two α subunits combine with 1 β subunit and one γ subunit to form a channel whose 3-dimensional structure allows Na^+ through a central pore. To be sure, octomeric and nonameric structures also have been proposed as the structural basis for the channel with similar functional implications.¹⁸ The topology of an individual subunit encompasses two transmembrane domains with the N- and C- termini of the polypeptide chain inwardly situated. The cytoplasmic portion of the C-terminus contains protein-protein binding domains such as the PY motif, where 2 prolines are arrayed to the N-terminal side of a tyrosine according to the sequence: x-Pro-Pro-x-Tyr.

ENaC increases net Na^+ transport across the apical membrane surface by either increasing the number channels on the cell surface or improving the probability that the channel's pore is open. Both mechanisms are regulated physiologically, with the preponderance of evidence suggesting that aldosterone governs ENaC cell surface expression and partially affects pore open probability.¹⁹ ENaC is delivered to the cell surface from subapical membrane vesicles via clathrin-mediated exocytosis.²⁰ ENaC is removed from the cell surface through ubiquitylation, directed by the protein Nedd4-2.²¹

Nedd4-2 is an ubiquitin ligase that initiates a cascade of enzymatic activity to link covalently the 76 amino acid polypeptide ubiquitin to specific peptide moieties on target proteins. The linkage allows the targeted protein to be degraded in proteasomes, or redirected to other subapical organelles. Nedd4-2 contains several tryptophan-rich regions termed WW domains. These WW domains bind with high affinity to PY motifs within the C-terminus of each of the ENaC subunits.

Data documenting immunofluorescent changes and amiloride-sensitive transmembrane potential differences in heterologous expression systems, such as *Xenopus laevis* oocytes, show the interaction between Nedd4-2 and ENaC subunits.²² Immunofluorescence and Na^+ transmembrane potential are reduced when oocytes are injected with RNA that

encodes for wild-type proteins. When either the PY domain of an ENaC subunit or the WW domain of Nedd4-2 is mutated, though, cell surface immunofluorescence and amiloride-sensitive current are restored.

The importance of the interaction between ENaC and Nedd4-2 can be seen clinically in Liddle's syndrome, an autosomal-dominant presentation of severe hypertension. Liddle's syndrome patients have been identified with specific mutations to the C-terminus of one of the ENaC subunits with a notable disruption of the PY motif.²³ Thus, Liddle's patients intrinsically are unable to downregulate ENaC from the apical membrane surface, yielding a gain-of-function phenotype for ENaC. It follows that these patients, in addition to having severe salt-sensitive hypertension, also will present with low serum aldosterone levels, low plasma renin activity, hypokalemia, and a metabolic alkalosis. Treatment is with triamterene or amiloride and a low-sodium diet. These patients are unresponsive to spironolactone, helping (along with the dominant inheritance pattern) to distinguish them from patients with AME.

The significance of exocytic processes governing cell surface expression of ENaC is less well understood. Cell culture studies implicate the aldosterone-responsive gene serum and glucocorticoid-induced kinase 1 (SGK1) indirectly in promoting clathrin-mediated exocytosis of ENaC subunits from subapical vesicles.²⁴ SGK1, a major secondary messenger at the interface between the molecular mechanism of aldosterone and ENaC, is detailed more broadly in the next section on aldosterone-sensitive genes.

Clues into the molecular handling of ENaC subunits also may be obtained through analysis of mutations that cause PHA1a, a rare autosomal-recessive syndrome caused by loss-of-function mutations to the α , β , or γ ENaC subunit.²⁵ PHA1a presents as a multisystem disease because ENaC also is critical in lung physiology. Thus, in addition to presenting like PHA1b patients with hypotension, sodium wasting, hyperkalemia, metabolic acidosis, and low serum aldosterone, PHA1a patients have severe respiratory problems that create a therapeutic challenge, especially during early childhood. Treatment often is difficult and includes significant sodium and fluid supplementation with supportive care.

NCC

The thiazide-sensitive sodium chloride cotransporter (NCC) is expressed in both parts of the distal convoluted tubule, DCT1 and DCT2. It is responsible for reabsorbing about 8% of filtered sodium. Regulation of NCC expression on the apical surface of renal tubular cells has received increasing attention over the past decade, in large measure because of the potency of thiazide diuretics in the treatment of essential hypertension, edema, and other cardiovascular diseases.²⁶ Scientific evidence now points to aldosterone as a regulator of NCC.²⁷ Data from animal studies has shown an increased abundance of NCC protein expression in hyperaldosterone states after exogenous administration of mineralocorticoids, low-sodium diets, or the extended use of loop diuretics.²⁸⁻³⁰ Microperfusion and histologic studies have shown that the

DCT2 segment has a large capacity to facilitate sodium reabsorption, an important finding because DCT2 (but not DCT1) expresses 11- β -HSD2 to allow for mineralocorticoid specificity.⁷ The extent to which aldosterone may increase NCC directly by a transcriptional mechanism, or via secondary messengers, remains unclear within these experimental models, underlining the critique that a more thorough understanding of how NCC is regulated by aldosterone still is needed. Scientific endeavors into NCC regulation have been hampered by the lack of sustainable primary or transformed cell lines expressing the transporter. Only animal models or heterologous expression systems (eg, RNA injections into *Xenopus laevis* oocytes) serve as the present models for study.

Gitelman's syndrome underscores the importance of NCC in renal sodium handling. Patients present as early as school age (5-18 years) with hypotension and electrolyte disturbances including hypokalemia, metabolic alkalosis, hypomagnesemia, and very low levels of urinary calcium excretion.³¹ The presentation mimics patients experiencing the untoward renal effects of thiazide overdose, with hypomagnesemia and hypocalciuria being the syndrome's distinguishing features in the differential diagnosis of other hyperreninemic, hyperaldosterone disorders. The syndrome results from point and frameshift mutations at numerous locations in the coding region of NCC.³²⁻³⁴ Many mutations result in improper processing of NCC within the Golgi apparatus, preventing their expression or orientation on the apical membrane surface. The protein products for the many mutations that cause Gitelman's syndrome are diverted into intracellular degradation pathways because the mutated proteins are not isolated in subapical vesicles. NCC thus appears to be a highly regulated gene product, perhaps helping to explain why no adequate tissue culture model systems have been developed.

Na,K,ATPase

Sodium reabsorption in the DCT2 is complete after Na⁺ has crossed the tubular epithelia. NCC and ENaC remove Na⁺ from the luminal filtrate into the cell's cytoplasm. NCC removes sodium and chloride from the filtrate in an electroneutral fashion, owing to a chemical gradient in which the luminal concentrations of the ions exceed the intracellular concentrations. ENaC regulates Na⁺ uptake under the influence of an electrochemical gradient. Both processes, however, are dependent on Na,K,ATPase active transport because this ubiquitous ion pump on the basolateral surface extrudes Na⁺ from the cell while setting up an electrochemical gradient with 3 sodium ions transferred from the cell to the plasma for every 2 potassium ions transported in the opposite direction.

The Na,K,ATPase also is regulated by aldosterone.³⁵ This regulation suggests a coordinated effort by the distal tubular cell to affect sodium transport by altering the transport systems for both membrane surfaces. The degree and mechanism by which Na,K,ATPase is regulated is time dependent. Aldosterone induces both short-acting (<3 h) and long-acting (>3 hours to days) effects on Na,K,AT-

Pase activity. Tissue culture and electrophysiologic studies in *Xenopus oocytes* corroborate observations in animal models that aldosterone increases Na,K,ATPase basolateral surface activity within each of these timeframes. The short-term effect appears to be mediated by SGK1 and increases basolateral surface expression of Na,K,ATPase subunits.³⁶ The process is dependent on SGK1 transcription, but not transcription of any Na,K,ATPase subunits; however, it remains unclear how SGK1 coordinates this increased basolateral pump expression. Long-term effects of aldosterone are seen through increased expression of the Na,K,ATPase pump's α subunit, leading to a more durable increase in pump activity during periods of prolonged hyperaldosteronemia.³⁷ No clinical correlates relevant to the kidney exist for genetic perturbations in Na,K,ATPase because the ubiquity of the pump would be expected to have more global consequences.

Aldosterone-Sensitive Genes: Kinases That Target Sodium Transporters

Thus far we have focused on the bookends of aldosterone's action to regulate transepithelial sodium transport in distal tubular cells. Beginning with the properties of 11- β -HSD2 and MR, it has been shown that the hormone initiates cellular responses with tissue specificity via largely transcriptional mechanisms. By summarizing what is known about how aldosterone influences the activity of the final targets—the transporters ENaC, NCC, and Na,K,ATPase—we have established that the hormone acts principally by indirect means. In this section the middle components of the pathway, the secondary messengers, are explored. Three secondary messengers—SGK1 and two WNK (With No K = lysine) kinases—are addressed in some detail whereas the direct induction by aldosterone of α ENaC and Na,K,ATPase are discussed briefly. To be sure, additional aldosterone-responsive genes, such as K-ras, have been identified and their significance should not be discounted.^{38,39}

SGK1

Over the past eight years, multiple scientific endeavors have established that SGK1 is an important mediator of the early effects (<3 h) of aldosterone.^{40,41} Originally cloned as a stress-induced gene from a tumor cell line, SGK1 is a serine-threonine kinase that is stimulated in many processes related to volume control.^{42,43} Whether in cell lines of collecting duct lineage or whole animals, aldosterone induces the transcription of SGK1 messenger RNA within 30 minutes. SGK1 protein levels increase with hormonal induction and stimulate ENaC-mediated Na⁺ transport within 1 hour. Although SGK1 abundance is dependent on mineralocorticoid exposure, SGK1 enzyme function requires specific activation by phosphatidylinositol-3 kinase (PI3K), an enzyme activated by insulin, among other mediators.^{44,45} Activated SGK1 phosphorylates specific serines and threonines of target proteins to alter their

function. In distal tubular epithelia, SGK1 phosphorylates Nedd4-2, reducing Nedd4-2 binding to ENaC subunits.²² This leads to an increase in cell surface expression of ENaC and augments net Na^+ transport because endocytosis of ENaC is impaired. The long-term effect of SGK1 is not sustained, however, because of a reciprocal activation/deactivation relationship between SGK1 and Nedd4-2.⁴⁶

In addition, SGK1 is implicated in other mechanisms that increase ENaC-mediated sodium transport. In a distal tubular cell model in which SGK1 levels can be altered artificially, the enzyme's actions include regulating ENaC open probability and augmenting channel subunit abundance via exocytic processes.²⁴ Further studies will need to be performed to clarify these observations, including identifying specific SGK1 protein targets.

During the early phase of aldosterone's action, SGK1 also increases Na,K,ATPase activity on the basolateral surface. This observation has been shown in both distal tubular cell lines and adrenalectomized rats, with support from experiments in *Xenopus oocytes* that the mechanism occurs through increased expression of Na,K,ATPase on the basolateral surface.⁴⁷⁻⁴⁹ Whether SGK1 targets Na,K,ATPase or works through other proteins is not understood. Taken together, ENaC and Na,K,ATPase are targets of the early phase of aldosterone's action via the functions of SGK1, suggesting that some degree of parallel response occurs by both tubular epithelial surfaces.

The later phase (>3 hours to days) effects of aldosterone on ENaC or Na,K,ATPase do not appear to depend on SGK1 to the same degree as the early phase. There may be some lingering effects from the enzyme, however, because the knockout transgenic mouse presents with hypotension and a salt-wasting phenotype when treated with a low-sodium diet.⁵⁰ More likely, aldosterone governs its long-term effects in part by directly inducing transcription and translation of both transporters.

WNK Kinases

WNK (With No K = lysine) kinase is another family of serine-threonine kinases. The name is derived from the unique characteristic of the kinase domain's catalytic component being absent a particular lysine residue that is found in other kinases. In 2001, mutations to WNK1 and WNK4 were shown to be the genetic determinants of the familial hypertensive trait pseudohypoaldosteronism type 2 (PHA2), or Gordon's syndrome.⁵¹ Patients with PHA2 have symptoms that include hypertension, hyperkalemia, and metabolic acidosis. Thiazide diuretics effectively treat PHA2, suggesting that WNK1 and WNK4 regulate NCC in some manner.

Subsequent studies have shown that WNK4 has multiple effects on transporter systems. Immunostaining of kidney tissue confirms the presence of WNK4 in the distal nephron segments DCT1, DCT2, connecting tubules, and cortical collecting duct.⁵¹ Expression is seen in both the cytoplasm and at the tight junctions, near the site of paracellular chloride transport. *Xenopus laevis*, oocytes WNK4 regulates the renal

outer medullary potassium channel (ROMK), paracellular chloride permeability, and the thiazide-sensitive NCC.⁵²⁻⁵⁴ In this heterologous expression system, WNK4 inhibits NCC-mediated Na^+ transport by more than 70% of function by reducing the cell surface expression of NCC. Although WNK4 has some autophosphorylation properties, the underlying mechanism of this inhibition does not appear to be caused by direct phosphorylation of NCC by WNK4. Instead, part of the inhibitory mechanism is a result of protein-protein binding between NCC and WNK4, as shown by immunoprecipitation studies.⁵⁵ Because some of the WNK4 mutations that cause PHA2 do not inhibit NCC-mediated sodium transport, their structure may provide clues to other effectors involved in the disease's pathogenesis.

WNK1 also is expressed in distal nephron tissue, but in the kidney two isoforms of it have been isolated, the wild-type (L-WNK1) and the so-called *kidney specific* form (ks-WNK1).⁵⁶ The two are distinguished genetically by having separate promoter regions and N-termini. In oocyte expression models, L-WNK1 does not directly affect NCC-mediated sodium transport, but it does prevent WNK4 inhibition of NCC by binding to WNK4.⁵⁵ Because mutations in WNK1 causing PHA2 are found in the gene's first intron, it is surmised that these mutations lead to overexpression of L-WNK1 and a hypertensive phenotype from excessive sodium reabsorption. How ks-WNK1 is involved in this process presently remains unexplained.

Recent work has shown that ks-WNK1, but not L-WNK1, is aldosterone sensitive.⁵⁷ Tissue culture studies confirmed that ks-WNK1 levels are increased in response to physiologic levels of aldosterone. These studies also confirmed that ks-WNK1 enhances ENaC-mediated Na^+ transport, with the mechanism affecting ENaC activity likely similar to that of SGK1, in that it affects channel trafficking from, and possibly to, the membrane surface.

Research studies on WNK1 isoforms and WNK4 are in their infancy. The genes' linkage to genetic forms of hypertension and their interactions with sodium transporters affected by aldosterone make them promising candidate genes to help decipher the pathogenesis of salt-sensitive, or other forms of, essential hypertension. In one Japanese study an association between WNK4 and essential hypertension was reached.⁵⁸ These genes also may be plausible candidates that help unravel the "aldosterone paradox"; that is, these genes may help explain the hormone's competing functions to restore body volume via sodium reabsorption pathways and to augment kaluresis by affecting ROMK.⁵⁹ Direct demonstration of this hypothesis still is needed, but indirect supportive evidence includes the following: WNK1 isoforms in kidney tissue are expressed and regulated differentially, including in vitro evidence that L-WNK1 modulates SGK1; protein-protein interaction between WNK1 and WNK4 alters sodium reabsorptive mechanisms; and the WNK1 isoforms and WNK4 have varied and differential effects on sodium, chloride, and potassium distal nephron transporters.

Table 1 Inherited Blood Pressure Disorders of the Aldosterone-Sensitive Distal Nephron

Disorder	Genetic Mutation	Mode of Inheritance	Clinical Characteristics	Molecular Pathway Derangement
AME	11- β -hydroxysteroid-dehydrogenase type 2 (11 β HSD-2)	Autosomal recessive	Hypertension, hypokalemia, metabolic alkalosis, low renin activity, low aldosterone, responsive to thiazides or spironolactone	Inactive 11 β HSD-2 allows cortisol to regulate sodium reabsorption by binding and activating the MR; loss of epithelial specificity to aldosterone-sensitive distal nephron
Gitelman's syndrome	Thiazide-sensitive NCC	Autosomal recessive	Hypotension, hypokalemia, metabolic alkalosis, hypomagnesemia, low urine calcium, high renin activity, high aldosterone levels	Loss of function to apical sodium chloride cotransporter
Liddle's syndrome	ENaC	Autosomal dominant	Hypertension, hypokalemia, metabolic alkalosis, low renin activity, low aldosterone, responsive to thiazides and triamterene	ENaC gain of function: mutations to c-terminus of ENaC subunits prevent Nedd4-2 ubiquitin ligase from removing ENaC from apical surface
PHA1a	ENaC	Autosomal recessive	Multisystem disease including hypotension, renal sodium wasting, hyperkalemia, metabolic acidosis, low renin, low aldosterone levels	Loss of function to ENaC including the lungs, kidneys, and colon
PHA1b	MR	Autosomal dominant	Hypotension, renal sodium wasting, hyperkalemia, metabolic acidosis, low renin, low aldosterone levels	Loss of function to the MR
PHA2 or Gordon's syndrome	WNK1 WNK4 Unknown gene	Autosomal dominant	Hypertension, hyperkalemia, hyperchloremic metabolic acidosis, low renin, low aldosterone, hypercalciuria	Increased expression of WNK1 caused by intron 1 mutation, thought to inhibit WNK4 or increase ENaC activity; WNK4 mutations alter WNK4 sequence, thought to alter WNK4 effects on sodium, potassium, or chloride transporters

Future Challenges: Aldosterone Regulation of Renal Sodium Handling

Guyton⁶⁰ posited that the mechanisms that underlie how the kidney regulates sodium excretion dominate long-term human

blood-pressure control. Perturbations in these processes that result in greater sodium retention, it followed, lead to hypertension and associated cardiovascular sequelae. Over the past half a century, aldosterone and the mechanisms it unleashes in the distal nephron critically affect volume homeostasis. This report sought to identify many of the known cellular targets of aldoste-

rone and characterize their functions in controlling sodium reabsorption (Fig 1). It incorporated Mendelian forms of hypertension and hypotension into the discussion to highlight the potential significance of these cellular pathways into the etiology of essential hypertension (Table 1). It also showed how simple medical observations can be integrated with physiologic, molecular, and genetic studies to improve our understanding of complicated diseases.

Progress into comprehending aldosterone's action on renal sodium handling has accelerated with improvements in biotechnology and represents a subtext of the review. The task over the next few decades will be to integrate these methodologies with advances in information technology to answer real biological questions about human disease. With a dizzying array of accelerated and promising tools for investigation—such as functional genomics, translational biology, computerized drug design, or bioinformatics—there is a danger that an avalanche of information will overwhelm and obscure the major investigative goals. The core challenge, thus, is to define the scientific objectives clearly.

For research into aldosterone and its role in sodium homeostasis, the central objective of the scientific endeavors is to apply findings about these mechanisms toward the diagnosis and treatment of hypertension and related diseases. With medical science expected to transform toward a more individualized approach to therapy, diagnosis based on an understanding of a patient's genomic or proteomic make-up will guide treatment regimens. The specific biological challenges in the study of aldosterone and sodium regulation are foremost:

1. **To identify the coregulators in the pathways that activate each sodium transporter.** Advances in identifying proteins from protein complexes or genetic microarrays will help in their isolation. Many of these coregulators will become candidate genes for medical geneticists to study in family or twin cohorts.
2. **To improve cell model systems to study the regulatory pathways of the sodium transporters.** Although the basic biology of ENaC and Na,K,ATPase have benefited from transformed cell lines, there is a paucity of such cells serving NCC biology, and an inadequate usage of primary cultures for each of the transporters. Stem cell research holds promise toward supplying these model systems. A corollary challenge with these cell models will be to better define the mechanisms involved in the longer term (>3 h) effects of aldosterone.
3. **To continue to apply physiological principles toward delineating aldosterone's mechanisms.** To some, transgenic mice models already are yesterday's technology, but it cannot be emphasized enough that the basic principles of sodium transport must be tested within the context of renal physiology, in which compensatory mechanisms by other nephron segments can have profound or unintended consequences.
4. **To interface co-regulators linked to hypertension into drug design or behavioral modification.** Sim-

plification and efficacy of therapeutic interventions will require identifying new agonists and antagonists that target coregulators in the aldosterone-induced pathway and formulating drug combinations that conform with a patient's genetic or proteomic profile. Although less entrepreneurial, these same profiles may aid in directing a patient toward specific lifestyle changes. For example, after analysis of the genetic profile, a particular patient may be directed to emphasize a high-potassium diet, rather than a low-sodium diet.

The advances made in the study of aldosterone action within the distal nephron have been impressive over the past half-century. As the evidence has mounted that the mechanisms the hormone unleashes affect human blood pressure, the components of these pathways have formed a core focus in the study of human hypertension. Clarification of the many scientific queries will benefit millions of people genetically predisposed to hypertension and its sequelae.

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