Sodium Transporters in the Distal Nephron and Disease Implications

Carolyn A. Ecelbarger, PhD, and Swasti Tiwari, PhD

Corresponding author
Carolyn A. Ecelbarger, PhD
Department of Medicine, Georgetown University, Box 571412, Washington, DC, 20057-1412, USA.
E-mail: ecelbarc@georgetown.edu

Current Hypertension Reports 2006, **8**:158–165
Current Science Inc. ISSN 1522-6417

The post–macula densa segments of the renal tubule—that is, the distal convoluted tubule, connecting tubule, and collecting duct—play a central role in determining final urine sodium excretion. The major regulated sodium transporters and channels in these cell types include the thiazide-sensitive (Na-Cl) cotransporter (NCC), the epithelial sodium channel (ENaC), and Na-K-ATPase. Furthermore, although not involved in sodium reabsorption, the anion exchanger, pendrin, and the basolateral bumetanide-sensitive Na-K-2Cl cotransporter (NKCC1 or BSC2) have roles in blood-volume maintenance. Mutations in several of these major sodium transporters, channel subunits, and their regulatory proteins have been linked to human diseases such as Liddle's syndrome, Gitelman's syndrome, and Gordon's syndrome, emphasizing the need for appropriate regulation of sodium at these sites for maintenance of sodium balance and normotension.

Introduction

It has been demonstrated that hypertension travels with the kidney when this organ is transplanted from a rat with genetic hypertension into a normotensive rat [1]. Furthermore, if one is to adopt the point of view that dysregulation of sodium transport in the kidney plays an important role in the development or maintenance of this hypertension, then the role of the distal tubule and collecting duct is of special concern. This may seem somewhat counterintuitive. Of the total filtered sodium load, approximately 65% is reabsorbed in the proximal tubule, 25% is reabsorbed in the thick ascending limb, and the remaining 10% is reabsorbed in the post–macula densa segments of the tubule—that is, the distal convoluted tubule, connecting tubule, and collecting duct [2]. Nearly 100% of the total filtered fraction of sodium is reabsorbed. However, it is the regulation of the final 10% (reabsorbed in post–macula densa cell types) that is most dynamic and suspect to change as a result of differences in dietary sodium intake. This regulation is primarily accomplished via alterations in the activity of the renin–angiotensin–aldosterone axis, with subsequent upregulation or downregulation of the activity of the sodium transporters and channels that are expressed in these distal cell types. One "cause" of hypertension is thought to be increased reabsorption of sodium in these distal sites. Additionally, several mutations of distal tubule sodium transporters, channel subunits, or regulators of transporters have been linked to disturbances in blood pressure regulation in humans [3]. Understanding what is known about the regulation of these select transporters and channels at a molecular level and how they may impact on blood pressure regulation and disease is the main focus of this review.

Cell Types in the Distal Nephron

The cell types that we focus on in this review include those that follow the macula densa cells with regard to direction of urine flow: 1) the distal convoluted tubule cells (DCT), of which there are two subclassifications (DCT1 and DCT2); 2) the connecting tubule cells (CNT); and 3) the collecting duct cells (CD) [4]. The CD extends from the cortex, where there are cortical collecting duct cells (CCD), through the outer medulla (OMCD), and finally into the inner medulla (IMCD). The collecting duct itself consists of two main categories of cell types: principal cells and intercalated cells of which there are α- and β-subtypes (or "A" and "B") and additional minor variant cell types ("non-A, non-B" for example). Principal cells are present throughout the CD, whereas intercalated cells are located mainly in the CCD and OMCD.

The distal convoluted tubule

The DCT (Fig. 1) reabsorbs approximately 5% to 7% of the filtered load of sodium, and plays an important role in the fine adjustment of urinary Na^+, K^+, and Ca^{2+} excretion [2]. Along the entire distal nephron, active sodium extrusion from the epithelial cell into the interstitium is carried out by the Na-K-ATPase pump, which is expressed on the basolateral aspect of these epithelial cells, and is essential for transepithelial sodium reabsorption. Na-K-

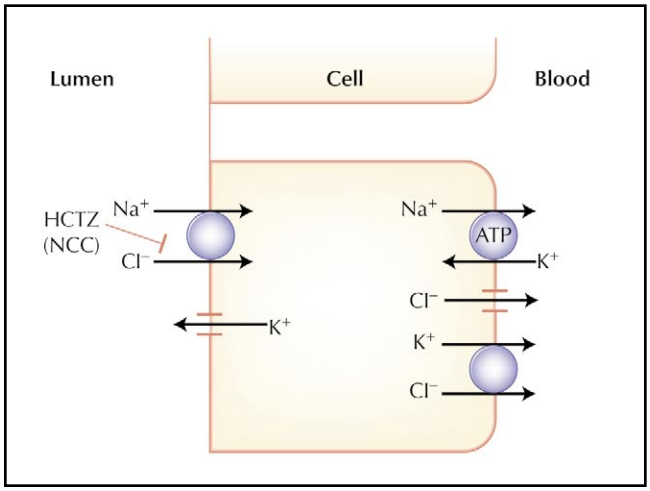

Figure 1. Cellular model for ion transport in the distal convoluted tubule (DCT). HCTZ—hydrochlorothiazide.

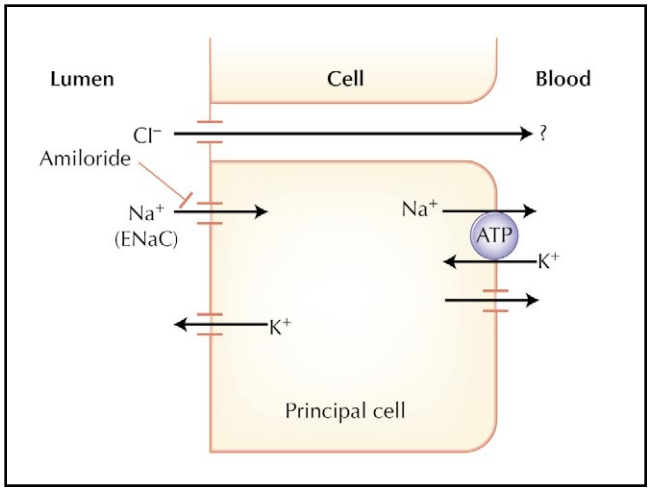

Figure 2. Cellular model for ion transport in the principal cells of the collecting duct. ENaC—epithelial sodium channel.

ATPase is an α-β complex, where α is the catalytic and transporting subunit [5]. Binding studies in rabbit tissue using tritiated ouabain showed the greatest density of Na-K-ATPase in the DCT (20–30 fmol/mm) followed by proximal tubule and thick ascending limb. Density in the medullary CD was significantly less (2–7 fmol/mm) [6].

Electroneutral absorption of Na^+ and Cl^- across the apical membrane of the distal convoluted tubule is accomplished by the thiazide-sensitive NaCl cotransporter (NCC or TSC) [7,8]. In the early 1990s, this protein was cloned from flounder, and subsequently from rat, mouse, rabbit, and human kidneys. Localization of NCC at the mRNA level, as well as at the protein level, using specific polyclonal antibodies [9] has demonstrated that expression of NCC in the rat kidney is confined to the DCT comprising the two subsegments, DCT1 and DCT2. DCT1 and DCT2 have subtle differences. In mouse and rat, they can be distinguished by the expression of several marker proteins—for example, DCT2 expresses calbindin-D and DCT1 does not. Studies using immunoelectron microscopy revealed NCC at the apical cell membrane and in the subapical cytoplasmic vesicles [9]. Besides NCC, there is another apical electrogenic sodium transport pathway present in DCT2 of rat kidney—the amiloride-sensitive epithelial sodium channel (ENaC), discussed later.

The connecting tubule and collecting duct

In most species, the cell type CNT is transitional between DCT and CD cell types. Apical sodium reabsorption in these segments is achieved via transport through ENaC, which is expressed only in the principal cell type (Fig. 2). In the kidney, ENaC consists of three subunits, α-, β-, and γ-ENaC, assembled as either a tetramer of two alphas, one beta, and one gamma subunit [10] or a higher ordered oligomer, such as a nonomer with three of each subunit [11]. ENaC has been localized at the mRNA and protein level in the CNT, CCD, OMCD, and IMCD, although some discrepancy exists as to its presence in the IMCD [12]. The sodium conductance through ENaC pores is sensitive to blockade by amiloride and its derivatives (eg, benzamil and triamterene).

Although they do not directly reabsorb sodium, the intercalated cells (Fig. 3) have a critical role in body fluid maintenance. Type B intercalated cells reabsorb chloride in exchange for various ions via pendrin (Pds), a recently cloned member of the SLC26 anion exchanger superfamily [13]. Pds has been shown to mediate Cl^-/iodide, Cl^-/formate, Cl^-/OH^-, and Cl^-/HCO_{3-} exchange activity when expressed in *Xenopus laevis* oocytes and HEK-293 cells. In the kidney, Pds is expressed in the distal nephron from DCT through CD [14]. Within these segments, Pds localizes to the apical plasma membrane and near apical cytoplasmic vesicles of both type B and non-A, non-B intercalated cells.

No overt renal abnormalities have been observed in Pds-null mice [15•] or in patients with mutations for Pds [16], that is, those with Pendred syndrome, which is, however, associated with deafness. Anion exchange has been demonstrated to be increased by aldosterone analogues in the collecting duct. Recently, Verlander et al. [15•] have shown that this effect may be mediated by increased apical targeting of Pds. In support of this, Pds has also been shown to be upregulated with NaCl restriction [17] and likely plays a role in chloride conservation. Pds-null mice have a lower apparent vascular volume and lower blood pressure under conditions of dietary sodium restriction [17]. In addition to this, Pds is also upregulated via aldosterone-independent mechanisms, such as acid-base status and chloride balance.

Also expressed in intercalated cells (type A) is the bumetanide-sensitive Na-K-2Cl cotransporter, BSC2 or NKCC1, also known as "the secretory isoform," as opposed to the thick ascending limb NKCC2 or BSC1. The gene, localized to mouse chromosome 18, encodes a widely expressed basolateral Na-K-2Cl cotransporter that was isolated from the shark rectal gland, a human

colonic cell line, and a mouse inner medullary CD cell line (mIMCD-3). In kidney, NKCC1 not only localizes to intercalated cells but has also been found in the juxtaglomerular afferent arteriole, as well as the glomerular and extraglomerular mesangium [18,19], where it is thought to participate in the process of tubuloglomerular feedback and the regulation of blood pressure. In addition, expression of NKCC1 along the basolateral membrane of rat renal intercalated cells suggests that the cotransporter also has a role in renal ammonium and/or acid secretion by this cell type [18]. Sodium and chloride are brought into the cell in the ratio of one to two on the cotransporter, whereas potassium exits to maintain electroneutrality. Eventually, the chloride is secreted into the lumen across the apical membrane of the cell. Thus, genetic disruption of NKCC1 would be predicted to reduce chloride secretion along the CD, leading to volume expansion and, possibly, hypertension. However, NKCC1-null mice show hypotension rather than hypertension [20], suggesting a renal defect in NaCl and water handling. Recently, Wall et al. [21•] have shown that in addition to vascular changes, NKCC1-null mice have diminished aldosterone release from the adrenal cortex as well as defects within the kidney that blunt the renal response to vasopressin and aldosterone, resulting in abnormal regulation of sodium, potassium, and water excretion. In addition, NKCC1 may also participate in the process of fluid secretion along the CD during physiologic and pathophysiologic states, such as with cyst formation in polycystic kidney disease [22].

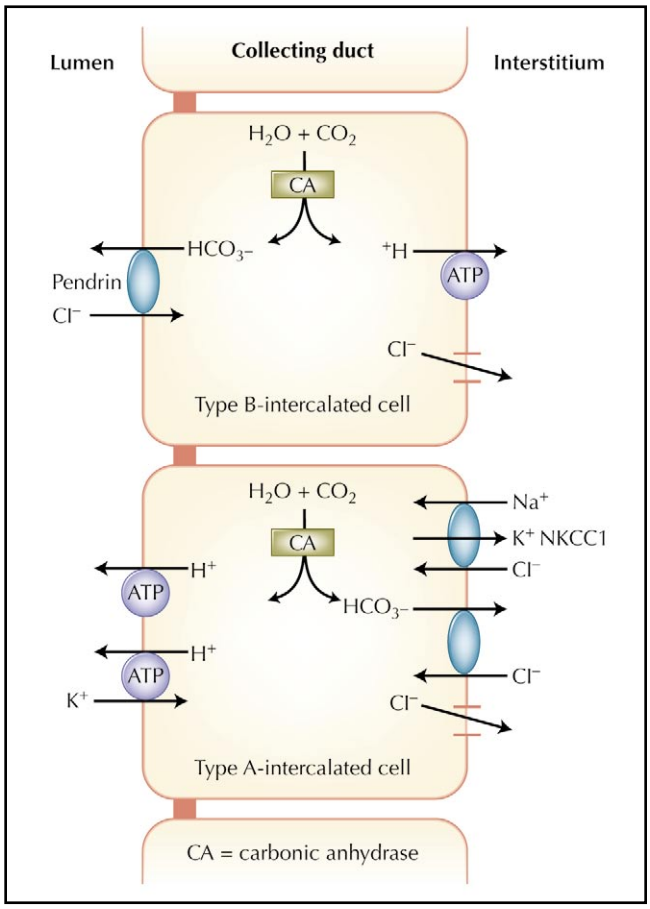

Figure 3. Cellular model for ion transport in the type A and type B intercalated cells of the collecting duct. HCO_{3^-}—bicarbonate; NKCC—Na-K-2CL cotransporter.

Regulation of Distal Tubule Sodium Transport

Regulation of distal tubule and CD sodium reabsorption is primarily under the regulation of the renin-angiotensin-aldosterone system (RAAS). However, other hormones have been shown to regulate at least ENaC activity; these include insulin and vasopressin. In addition, stimulation of the renal sympathetic nervous system may affect renal sodium reabsorption in the distal tubule and CD via direct action at adrenoreceptors [23]. Activity of the specific transport proteins can be regulated in a variety of ways, including: 1) changes in whole cell, and presumably active protein levels; 2) subcellular redistribution or trafficking of these transporters and channels from nonactive cytoplasmic domains into the apical or basolateral membrane where they are inserted and become integral and active membrane proteins; 3) gating or post-translational modification or activation of existing plasma membrane proteins—for example, proteolytic cleavage or phosphorylation events.

Aldosterone

Aldosterone is the major regulator of day-to-day sodium reabsorptive activity in the distal tubule and collecting duct. These cell types express both mineralocorticoid receptors and the enzyme 11β-hydroxysteroid dehydrogenase 2 (11-β-HSD-2) [24], which converts cortisol into inactive cortisone, a prerequisite for an "aldosterone-sensitive" tissue. In addition, perfused tubule studies have shown that aldosterone or mineralocorticoid analogues increase sodium reabsorption in the kidney at these sites. One protein upregulated by aldosterone is the basolateral Na-K-ATPase pump [25].

Furthermore, recently Knepper's group [26,27] showed that aldosterone infusion into rats increased the protein abundance of NCC [27] and the α-subunit of ENaC [26]. However, the β-subunit was either decreased or not changed, and the γ-subunit underwent a chemical change that altered its appearance on immunoblots—that is, there was a decreased band density of the major band (85 kDa) and increased band density of a lower molecular weight set of bands or a band region around 70 kDa. The chemical nature of this band region is yet unknown, although it may represent activating proteolysis. Aldosterone infusion also resulted in redistribution of ENaC from subapical domains into the apical plasma membrane in rat kidney [26]. However, redistribution of NCC with aldosterone infusion has not yet been demonstrated.

In addition to aldosterone infusion, feeding rats a low-NaCl diet resulted in many of the same molecular changes in ENaC and NCC [26,27]. The role of the mineralocorticoid receptor (MR) in this response was confirmed by additional studies by Nielsen et al. [28] showing reversal of the upregulation of NCC, α-ENaC, and the 70-kDa band of γ-ENaC in response to a low-NaCl diet with concomitant administration of spironolactone, a mineralocorticoid receptor antagonist in the diet [28]. Furthermore, dietary NaCl restriction increased mRNA expression of α-ENaC, but not β- or γ-ENaC, nor NCC [29] in rat kidney, suggesting that the aldosterone-mediated increase in renal NCC protein abundance occurs by an alternative mechanism to mRNA regulation, such as the regulation of protein degradation or mRNA expression rate or stability.

On the other hand, distal tubule sodium transporters are downregulated in abundance and, likely, activity in models of "aldosterone escape." Aldosterone escape is a model of pressure natriuresis in which aldosterone levels are clamped high, and dietary NaCl is switched from low NaCl to high NaCl [30]. In this situation, there is marked downregulation of NCC and the 70-kDa band of γ-ENaC, compared to the "control rats"—that is, rats infused with aldosterone and maintained on the low-NaCl diet [30]. Thus, this represents a mode of regulation of these "aldosterone-sensitive proteins" that is clearly independent of the circulating level of aldosterone. Nitric oxide is proposed to play a role in the escape, as the nonspecific nitric oxide synthase inhibitor, L-NAME (NG-nitro-L-arginine methyl ester) treatment of escaping rats was shown to reverse several protein changes [31].

Angiotensin II (Ang II)

Although it is clearly upstream from aldosterone in the RAAS signaling cascade, Ang II has been shown to regulate distal tubule sodium transporters independent of aldosterone [32]. This is accomplished via binding to G-protein coupled Ang II receptors (AT1 and AT2) expressed in the DCT and CD cells. AT1 receptors mediate sodium reabsorption and AT2, natriuresis. Rats, mice, and humans express two isoforms of AT1: AT1a and AT1b. Thus, part of the adaptation to low dietary sodium intake—increased abundance of NCC and α-ENaC—is likely mediated by increased Ang II levels. This hypothesis is supported by Brooks et al. [33], who found decreased upregulation of NCC and α-ENaC in AT1a receptor–null mice in response to low-NaCl diet, despite a significant increase in circulating aldosterone levels, relative to wild-type mice.

Vasopressin

Vasopressin, a nine–amino acid peptide, has a role not only in water reabsorption through its actions in the CNT and CD but it also increases sodium reabsorption via direct activation of ENaC. Vasopressin, or its analogues, applied acutely increased sodium reabsorption in perfused tubules from rat and rabbit, in rabbit CCD suspensions, in M-1 cells derived from mice CCD and in A6 cells from toad bladder [34]. Vasopressin V2 receptors (G-protein coupled), which mediate sodium-retentive actions of vasopressin, have been localized in the distal nephron in the CNT through the entire length of the CD, but apparently not in DCT.

Chronic vasopressin treatment of rats via either infusion of dDAVP (desmopressin, 1-deamino-8-D-arginine-vasopressin, a V2-receptor-selective agonist of vasopressin) into Brattleboro rats, which lack endogenous vasopressin, or by water restriction of Sprague-Dawley rats (raises endogenous vasopressin) has been shown to result in increased protein abundance for the β- and γ-subunits of ENaC, with no consistent effect on the α-subunit [35]. In agreement, chronic exposure of RCCD1 cells with vasopressin increased the expression of the mRNA for β- and γ-ENaC (but not α-), and this increase coincided with an increase in uptake of ^{22}Na in these cells [36]. Recently, a potent V2-dependent, antinatriuretic effect of infused dDAVP was demonstrated in humans, which raises the possibility that an inappropriate stimulation of ENaC by vasopressin might lead to significant sodium retention in disease states with inappropriately high vasopressin levels [37].

We have also shown that NCC, the ENaC subunits, are regulated in the model of vasopressin escape [38,39]. In this model, rats are administered a continuous infusion of dDAVP and given a liquid diet as their sole food source. This encourages them to drink more water than they normally would, to get the calories they desire. The rats transiently retain water and then go through a natriuresis, followed by a diuresis, "vasopressin escape." This escape is associated with a strong upregulation of α-ENaC, NCC, and the lower band of γ-ENaC (70 kDa), which in other studies has been shown to be regulated by aldosterone [26,27]. Subsequent studies showed increased circulating levels and urinary excretion of aldosterone in escape, although our more recent work (unpublished) suggests that these changes may be independent of the circulating level of aldosterone. This model, interestingly, also is associated with increased blood pressure [38].

Insulin

The hormone insulin may play a role in the development of the hypertension associated with "syndrome X" or "the metabolic syndrome," a common age-related syndrome that is expressed as hyperinsulinemia, visceral adiposity, hypertension, and lipid abnormalities. During the past several decades, a bulk of information has accumulated indicating that physiologic changes in plasma insulin concentrations are capable of affecting electrolyte transport by the kidney. Early studies by Atchley et al. [40] demonstrated that discontinuation of an insulin infusion to diabetic patients resulted in a brisk natriuresis. Furthermore, studies showed that infusion of insulin to

Table 1. Monogenetic diseases and affected sodium transporter/channel

Syndrome	Segment of nephron	Transporter/ channel affected	Gene mutated	Chromosome	Inheritance
Apparent mineralocorticoid excess (AME)	Distal nephron	NCC/ENaC	*HSD*11K	16q22	Recessive
Pseudohypoaldosteronism 1 (PHA 1)	CD	α, β, and γENaC	*SCNN1A*, *SCNN1B*, *SCNN1G*	16p13-p12, 12p13	Recessive
Liddle's syndrome	CD	βENaC, γENaC	*SCNN1B*, *SCNN1G*	16p13-p12	Dominant
Gitelman's syndrome	DCT	NCC	*SLC*1243	16q13	
Gordon's syndrome	DCT	NCC	*WNK1* and *WNK4*	7q11-21	Dominant

CD—collecting ducts; DCT—distal convoluted tubule cells; ENaC—epithelial sodium channel; NCC—NaCl cotransporter.

normal subjects resulted in a reduction in urinary sodium excretion. More detailed studies by DeFronzo et al. [41,42] demonstrated that, in both dogs and humans, insulin increased sodium transport in the kidney, as well as reduced sodium excretion, independent of blood-glucose levels, filtered load of glucose, GFR, renal blood flow, and plasma aldosterone levels.

Micropuncture and perfused tubule studies have revealed that insulin increases sodium reabsorption in the distal tubule or collecting duct. Insulin receptors, as measured by in situ binding with ^{125}I-labeled insulin, have been localized along the entire length of the renal tubule with density apparently the highest in the thick ascending limb and in the distal convoluted tubule. In the distal part of the tubule, insulin has been reported to activate both Na-K-ATPase [43,44], probably by phosphorylation, and the amiloride-sensitive epithelial sodium channel, ENaC. ENaC activation by insulin in cell culture results from an increase in the density of ENaC channels on the membrane.

We and our colleagues [45] have shown that renal NCC, β-ENaC, and the α-1 subunit of Na-K-ATPase are increased in protein abundance in the insulin-resistant, hyperinsulinemic, obese Zucker rat, relative to lean age-mates. In more recent studies, we showed that NCC activity and blood pressure were decreased when these rats were treated with an insulin-sensitizing drug, the peroxisomal proliferator activated receptor (PPAR) subtype γ agonist, rosiglitazone [46], which reduced circulating levels of insulin. However, neither NCC kidney abundance nor urinary excretion levels, which were found elevated in obese rats, were reduced by the rosiglitazone.

Other regulators of distal tubule sodium transporters and channels

NCC also has been shown to be upregulated by estradiol [47], which may help to explain the more pronounced diuresis in response to thiazides in female rats compared to male rats [48]. This contrasted with our own studies showing 17β-estradiol replacement (administered every fourth day to mimic the rat estrus cycle) to ovariectomized female rats resulted in a significant downregulation of whole kidney NCC abundance relative to ovariectomized rats without replacement [49]. However, in this study, 17β-estradiol replacement had no significant effect on the ENaC subunits.

Recently, ENaC has been shown to be inhibited by adenosine monophosphate–activated kinase (AMPK) [50]. AMPK is a ubiquitous metabolic-sensing serine-threonine kinase whose activity increases during conditions of metabolic stress—that is, in response to elevated intracellular AMP:ATP ratios. Carattino et al. [50] have shown that activation of AMPK inhibits ENaC activity in both the *Xenopus* oocyte expression system and in polarized mouse cortical CD (mpkCCD$_{c14}$) cells. They determined that AMPK stimulation decreases ENaC surface expression, presumably through effects on Nedd4-2–mediated endocytic retrieval. Nedd4-2 is a ubiquitin-protein ligase that regulates ENaC cell surface expression and is regulated by the aldosterone-stimulated serum and glucocorticoid kinase (sgk-1) [51]. Thus, AMPK-dependent regulation of ENaC may link ENaC activity to cellular metabolic status and have a role in inhibition of ENaC and natriuresis that occurs with metabolic depletion.

Monogenetic (Mendelian) Forms of Hypertension and Distal Tubular Sodium Transport

The critical role of the appropriate regulation of distal tubule sodium transporters and channels in blood pressure maintenance is highlighted by the fact that blood pressure is abnormal in several monogenetic mutations of specific transporters, channel subunits, or regulators of transport (Table 1). The demonstration of genetic linkage between a monogenic form of human hypertension—that is, Liddle's syndrome—supports a central role for ENaC in blood pressure regulation. In Liddle's syndrome, mutations in the genes on chromosome 16p12 that encode the β- and γ-subunits of ENaC, SCNNIB, and SCNNIG, respectively, have been found. These are, specifically, deletions or substitutions in a short proline-rich segment of the intracytoplasmic C-terminus, which result in the inability of these subunits to bind a protein that removes the epithelial sodium channel from the cell surface, with a resultant over-

expression of sodium channels and constitutively active sodium reabsorption. These particular "gain-of-function" mutations mimic the effects of hyperaldosteronism, and, therefore, are called pseudohyperaldosteronism. In addition, a "loss of function mutation" has also been described for ENaC. In pseudohypoaldosteronism type I (PHA-I) patients, a missense mutation allowing the substitution of glycine with a serine at position 37 in the amino tail of all three ENaC subunits (this is a conserved region common among all three subunits) results in markedly reduced sodium conductivity and, therefore, pseudohypoaldosteronism and hypotension [52]. Wong et al. [53] have recently shown that genetic variability in chromosome 16p12, which encodes the β- and γ- subunits of ENaC, explains a fair amount of the variability in systolic blood pressure in normal individuals using four highly polymorphic microsatellite markers.

Similarly, "loss-of-function" mutations in NCC are the cause of Gitelman's syndrome [54•]. This disease is characterized by urinary sodium loss, resulting in orthostatic hypotension. On the other hand, loss of function mutations in the enzyme 11β-hydroxysteroid dehydrogenase 2 (11-β-HSD-2), which converts cortisol into inactive cortisone in "aldosterone-sensitive" tissues, such as the distal nephron, results in the syndrome of "apparent mineralocorticoid excess" (AME), with severe hypertension and hypokalemia [55]. Dietary salt excess does not appear to affect 11-β-HSD-2 activity per se, but recently salt-sensitive hypertension has been associated with reduced 11-β-HSD-2 activity, and with a specific polymorphism in the *11-β-HSD-2* gene [56]. Additionally, mutations in *WNK1* and *WNK4*, genes encoding members of a novel family of serine-threonine kinases that are expressed in the DCT and the CD of kidney, have been shown to cause PHA-II, or Gordon's syndrome, an autosomal-dominant disorder featuring hypertension, hyperkalemia, renal tubular acidosis, and the dysregulation of ion transport in the distal nephron of the kidney [57•]. Activating mutations in two isoforms of the WNK kinase family (WNK1 and WNK4) expressed in the distal part of the nephron, and localized to either the cytoplasmic compartment (WNK1) or in the tight junctions (WNK4) of the tubular cells, have been demonstrated in patients with Gordon's syndrome [57•]. Evidence suggests that these mutations lead to inappropriately high and possibly constitutively active NCC activity [58]. Interestingly, the *WNK4* gene is located in a chromosomal region (17q12–21) that has been linked to blood pressure by several genome scans [59].

Chronic Renal Failure, Hypertension, and the Distal Nephron

Nephropathy is a primary factor that underlies the development of hypertension in type 1 diabetes. Diabetic nephropathy is commonly associated with renal salt and water retention and hypertension. Urinary tumor necrosis factor (TNF) is an important mediator of diabetic nephropathy that promotes distal tubule sodium retention and renal hypertrophy during diabetes [60]. Recently, it has been shown that chronic TNF exposure leads to distal tubule sensitization that permits acute TNF-induced activation of ENaC [60]. Also, diabetes is associated with diuresis and natriuresis, both of which can stimulate vasopressin and RAAS activity. We have observed upregulation of both NCC and ENaC in streptozotocin-treated diabetic rats after only 4 days of diabetes [61]. Upregulation of these transport pathways may attenuate sodium losses. In addition, patients with chronic renal failure often are prone to sodium retention. Recent clinical work [62] suggests that hydrochlorothiazide (targeting NCC) may be more effective than furosemide (targets TAL) in reducing inappropriate sodium retention, again implicating relatively greater dysregulation of the distal nephron in sodium retention.

Conclusions

The distal nephron plays a determining role in final excretion of filtered sodium. This is accomplished by fine-tuning of the major sodium reabsorptive proteins, which when active, line the renal apical and basolateral plasma membranes. These proteins are under tight regulation by several hormone systems, including the RAAS, vasopressin, and insulin. Mutations in the genes for these proteins highlight the importance of appropriate regulation of transport at these sites for maintenance of normal body fluid levels and prevention of disease states such as hypertension.

References and Recommended Reading

Papers of particular interest, published recently, have been highlighted as:

- • Of importance
- •• Of major importance

1. Bianchi G, Fox U, Di Francesco GF, et al.: **Blood pressure changes produced by kidney cross-transplantation between spontaneously hypertensive rats and normotensive rats.** *Clin Sci Mol Med* 1974, **47:**435–448.
2. Vander AJ: **Basic renal processes for sodium, chloride, and water.** In *Renal Physiology,* edn 5. New York: McGraw-Hill; 1995:89–115.
3. Lifton RP: **Molecular genetics of human blood pressure variation.** *Science* 1996, **272:**676–680.
4. Bachmann S, Kriz W: **Histology, cytology, ultrastructure. Nephron and collecting duct structure in the kidney rat.** In *Urinary System.* Edited by Jones TC, Hard GC, Mohr U. Heidelberg: Springer; 1998.
5. Lingrel JB: **Na,K-ATPase: isoform structure, function, and expression.** *J Bioenerg Biomembr* 1992, **24:**263–270.
6. El Mernissi G, Doucet A: **Quantitation of [3H]ouabain binding and turnover of Na-K-ATPase along the rabbit nephron.** *Am J Physiol* 1984, **247:**F158–F167.
7. Velazquez H, Bartiss A, Bernstein P, et al.: **Adrenal steroids stimulate thiazide-sensitive NaCl transport by rat renal distal tubules.** *Am J Physiol* 1996, **270:**F211–F219.

8. Ellison DH, Velazquez H, Wright FS: **Thiazide-sensitive sodium chloride cotransport in early distal tubule.** *Am J Physiol* 1987, **253:**F546–F554.
9. Plotkin MD, Kaplan MR, Verlander JW, et al.: **Localization of the thiazide sensitive Na-Cl cotransporter, rTSC1 in the rat kidney.** *Kidney Int* 1996, **50:**174–183.
10. Firsov D, Gautschi I, Merillat AM, et al.: **The heterotetrameric architecture of the epithelial sodium channel (ENaC).** *EMBO J* 1998, **17:**344–352.
11. Snyder PM, Cheng C, Prince LS, et al.: **Electrophysiological and biochemical evidence that DEG/ENaC cation channels are composed of nine subunits.** *J Biol Chem* 1998, **273:**681–684.
12. Duc C, Farman N, Canessa CM, et al.: **Cell-specific expression of epithelial sodium channel alpha, beta, and gamma subunits in aldosterone-responsive epithelia from the rat: localization by in situ hybridization and immunocytochemistry.** *J Cell Biol* 1994, **127:**1907–1921.
13. Everett LA, Green ED: **A family of mammalian anion transporters and their involvement in human genetic diseases.** *Hum Mol Genet* 1999, **8:**1883–1891.
14. Kim YH, Kwon TH, Frische S, et al.: **Immunocytochemical localization of pendrin in intercalated cell subtypes in rat and mouse kidney.** *Am J Physiol Renal Physiol* 2002, **283:** F744–F754.
15.• Verlander JW, Hassell KA, Royaux IE, et al.: **Deoxycorticosterone upregulates PDS (Slc26a4) in mouse kidney: role of pendrin in mineralocorticoid-induced hypertension.** *Hypertension* 2003, **42:**356–362.

This article is important, as it shows the upregulation of pendrin with aldosterone analogues, which could be critical in the pathogenesis of mineralocorticoid-induced hypertension and metabolic alkalosis.

16. Royaux IE, Wall SM, Karniski LP, et al.: **Pendrin, encoded by the Pendred syndrome gene, resides in the apical region of renal intercalated cells and mediates bicarbonate secretion.** *Proc Natl Acad Sci U S A* 2001, **98:**4221–4226.
17. Wall SM, Kim YH, Stanley L, et al.: **NaCl restriction upregulates renal Slc26a4 through subcellular redistribution: role in Cl- conservation.** *Hypertension* 2004, **44:**982–987.
18. Ginns SM, Knepper MA, Ecelbarger CA, et al.: **Immunolocalization of the secretory isoform of Na-K-Cl cotransporter in rat renal intercalated cells.** *J Am Soc Nephrol* 1996, **7:**2533–2542.
19. Kaplan MR, Mount DB, Delpire E: **Molecular mechanisms of NaCl cotransport.** *Annu Rev Physiol* 1996, **58:**649–668.
20. Meyer JW, Flagella M, Sutliff RL, et al.: **Decreased blood pressure and vascular smooth muscle tone in mice lacking basolateral Na(+)-K(+)-2Cl(-) cotransporter.** *Am J Physiol Heart Circ Physiol* 2002, **283:**H1846–H1855.
21.• Wall SM, Knepper MA, Hassell KA, et al.: **Hypotension in NKCC1 null mice: role of the kidneys.** *Am J Physiol Renal Physiol* 2005, Epub ahead of print.

This article is important, as it shows that the action of aldosterone and vasopressin is altered within kidneys of NKCC1 null mice, which likely contributes to their hypotension.

22. Lebeau C, Hanaoka K, Moore-Hoon ML, et al.: **Basolateral chloride transporters in autosomal dominant polycystic kidney disease.** *Pflugers Arch* 2002, **444:**722–731.
23. Cuffe JE, Howard DP, Bertog M, et al.: **Basolateral adrenoceptor activation mediates noradrenaline-induced Cl- secretion in M-1 mouse cortical collecting duct cells.** *Pflugers Arch* 2002, **445:**381–389.
24. Bostanjoglo M, Reeves WB, Reilly RF, et al.: **11Beta-hydroxysteroid dehydrogenase, mineralocorticoid receptor, and thiazide-sensitive Na-Cl cotransporter expression by distal tubules.** *J Am Soc Nephrol* 1998, **9:**1347–1358.
25. Vinciguerra M, Mordasini D, Vandewalle A, et al.: **Hormonal and nonhormonal mechanisms of regulation of the NA,K-pump in collecting duct principal cells.** *Semin Nephrol* 2005, **25:**312–321.
26. Masilamani S, Kim GH, Mitchell C, et al.: **Aldosterone-mediated regulation of ENaC alpha, beta, and gamma subunit proteins in rat kidney.** *J Clin Invest* 1999, **104:**R19–R23.
27. Kim GH, Masilamani S, Turner R, et al.: **The thiazide-sensitive Na-Cl cotransporter is an aldosterone-induced protein.** *Proc Natl Acad Sci U S A* 1998, **95:**14552–14557.
28. Nielsen J, Kwon TH, Masilamani S, et al.: **Sodium transporter abundance profiling in kidney: effect of spironolactone.** *Am J Physiol Renal Physiol* 2002, **283:**F923–F933.
29. Masilamani S, Wang X, Kim GH, et al.: **Time course of renal Na-K-ATPase, NHE3, NKCC2, NCC, and ENaC abundance changes with dietary NaCl restriction.** *Am J Physiol Renal Physiol* 2002, **283:**F648–F657.
30. Wang XY, Masilamani S, Nielsen J, et al.: **The renal thiazide-sensitive Na-Cl cotransporter as mediator of the aldosterone-escape phenomenon.** *J Clin Invest* 2001, **108:**215–222.
31. Turban S, Wang XY, Knepper MA: **Regulation of NHE3, NKCC2, and NCC abundance in kidney during aldosterone escape phenomenon: role of NO.** *Am J Physiol Renal Physiol* 2003, **285:**F843–F851.
32. Beutler KT, Masilamani S, Turban S, et al.: **Long-term regulation of ENaC expression in kidney by angiotensin II.** *Hypertension* 2003, **41:**1143–1150.
33. Brooks HL, Allred AJ, Beutler KT, et al.: **Targeted proteomic profiling of renal Na(+) transporter and channel abundances in angiotensin II type 1a receptor knockout mice.** *Hypertension* 2002, **39:**470–473.
34. Ecelbarger CA, Kim GH, Wade JB, et al.: **Regulation of the abundance of renal sodium transporters and channels by vasopressin.** *Exp Neurol* 2001, **171:**227–234.
35. Ecelbarger CA, Kim GH, Terris J, et al.: **Vasopressin-mediated regulation of epithelial sodium channel abundance in rat kidney.** *Am J Physiol Renal Physiol* 2000, **279:**F46–F53.
36. Djelidi S, Fay M, Cluzeaud F, et al.: **Transcriptional regulation of sodium transport by vasopressin in renal cells.** *J Biol Chem* 1997, **272:**32919–32924.
37. Bankir L, Fernandes S, Bardoux P, et al.: **Vasopressin-V2 receptor stimulation reduces sodium excretion in healthy humans.** *J Am Soc Nephrol* 2005, **16:**1920–1928.
38. Song J, Hu X, Khan O, et al.: **Increased blood pressure, aldosterone activity, and regional differences in renal ENaC protein during vasopressin escape.** *Am J Physiol Renal Physiol* 2004, **287:**F1076–F1083.
39. Ecelbarger CA, Knepper MA, Verbalis JG: **Increased abundance of distal sodium transporters in rat kidney during vasopressin escape.** *J Am Soc Nephrol* 2001, **12:**207–217.
40. Atchley D, Loeb RF, Richards DW, et al.: **On diabetic acidosis.** *J Clin Invest* 1933, **12:**297–326.
41. DeFronzo RA, Cooke CR, Andres R, et al.: **The effect of insulin on renal handling of sodium, potassium, calcium, and phosphate in man.** *J Clin Invest* 1975, **55:**845–855.
42. DeFronzo RA, Goldberg M, Agus ZS: **The effects of glucose and insulin on renal electrolyte transport.** *J Clin Invest* 1976, **58:**83–90.
43. Chibalin AV, Kovalenko MV, Ryder JW, et al.: **Insulin- and glucose-induced phosphorylation of the Na(+),K(+)-adenosine triphosphatase alpha-subunits in rat skeletal muscle.** *Endocrinology* 2001, **142:**3474–3482.
44. Feraille E, Rousselot M, Rajerison R, et al.: **Effect of insulin on Na+,K(+)-ATPase in rat collecting duct.** *J Physiol* 1995, **488:**171–180.
45. Bickel CA, Verbalis JG, Knepper MA, et al.: **Increased renal Na-K-ATPase, NCC, and beta-ENaC abundance in obese Zucker rats.** *Am J Physiol Renal Physiol* 2001, **281:**F639–F648.
46. Khan O, Riazi S, Hu X, et al.: **Regulation of the renal thiazide-sensitive Na-Cl cotransporter, blood pressure, and natriuresis in obese Zucker rats treated with rosiglitazone.** *Am J Physiol Renal Physiol* 2005, **289:**F442–F450.
47. Verlander JW, Tran TM, Zhang L, et al.: **Estradiol enhances thiazide-sensitive NaCl cotransporter density in the apical plasma membrane of the distal convoluted tubule in ovariectomized rats.** *J Clin Invest* 1998, **101:**1661–1669.

48. Chen Z, Vaughn DA, Fanestil DD: **Influence of gender on renal thiazide diuretic receptor density and response.** *J Am Soc Nephrol* 1994, **5:**1112–1119.
49. Riazi S, Maric C, Ecelbarger C: **17-beta estradiol attenuates streptozotocin-induced diabetes and regulates the expression of renal sodium transporters.** *Kidney Int* 2006, In press.
50. Carattino MD, Edinger RS, Grieser HJ, et al.: **Epithelial sodium channel inhibition by AMP-activated protein kinase in oocytes and polarized renal epithelial cells.** *J Biol Chem* 2005, **280:**17608–17616.
51. Flores SY, Debonneville C, Staub O: **The role of Nedd4/Nedd4-like dependant ubiquitylation in epithelial transport processes.** *Pflugers Arch* 2003, **446:**334–338.
52. Grunder S, Firsov D, Chang SS, et al.: **A mutation causing pseudohypoaldosteronism type 1 identifies a conserved glycine that is involved in the gating of the epithelial sodium channel.** *EMBO J* 1997, **16:**899–907.
53. Wong ZY, Stebbing M, Ellis JA, et al.: **Genetic linkage of beta and gamma subunits of epithelial sodium channel to systolic blood pressure.** *Lancet* 1999, **353:**1222–1225.
54.• Scheinman SJ, Guay-Woodford LM, Thakker RV, et al.: **Genetic disorders of renal electrolyte transport.** *N Engl J Med* 1999, **340:**1177–1187.
They have reviewed mutations affecting the ENaC and diuretic-sensitive sodium-transport proteins.
55. Mune T, White PC: **Apparent mineralocorticoid excess: genotype is correlated with biochemical phenotype.** *Hypertension* 1996, **27:**1193–1199.
56. Lovati E, Ferrari P, Dick B, et al.: **Molecular basis of human salt sensitivity: the role of the 11beta-hydroxysteroid dehydrogenase type 2.** *J Clin Endocrinol Metab* 1999, **84:**3745–3749.
57.• Wilson FH, Disse-Nicodeme S, Choate KA, et al.: **Human hypertension caused by mutations in WNK kinases.** *Science* 2001, **293:**1107–1112.
The authors establish that mutations in two members of the WNK family of serine-threonine kinases cause PHAII. Their finding may offer new targets for the development of antihypertensive drugs.
58. Yang CL, Angell J, Mitchell R, et al.: **WNK kinases regulate thiazide-sensitive Na-Cl cotransport.** *J Clin Invest* 2003, **111:**1039–1045.
59. Levy D, DeStefano AL, Larson MG, et al.: **Evidence for a gene influencing blood pressure on chromosome 17. Genome scan linkage results for longitudinal blood pressure phenotypes in subjects from the framingham heart study.** *Hypertension* 2000, **36:**477–483.
60. DiPetrillo K, Coutermarsh B, Soucy N, et al.: **Tumor necrosis factor induces sodium retention in diabetic rats through sequential effects on distal tubule cells.** *Kidney Int* 2004, **65:**1676–1683.
61. Song J, Knepper MA, Verbalis JG, et al.: **Increased renal ENaC subunit and sodium transporter abundances in streptozotocin-induced type 1 diabetes.** *Am J Physiol Renal Physiol* 2003, **285:**F1125–F1137.
62. Dussol B, Moussi-Frances J, Morange S, et al.: **A randomized trial of furosemide vs hydrochlorothiazide in patients with chronic renal failure and hypertension.** *Nephrol Dial Transplant* 2005, **20:**349–353.