CHAPTER

6 Tubular Potassium Transport

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TUBULAR POTASSIUM TRANSPORT

Potassium (K^+) functions in a diversity of physiologic processes. Changes in intracellular K^+ affect cell volume, intracellular pH, enzymatic function, protein synthesis, DNA synthesis, and apoptosis. Changes in the ratio of intracellular to extracellular K^+ affect the cellular resting membrane potential, causing depolarization in hyperkalemia and hyperpolarization in hypokalemia; as a consequence, potassium disorders primarily affect excitable tissues, chiefly heart and muscle. Hypokalemia and hyperkalemia also have a variety of renal and cardiovascular consequences. A large body of experimental and epidemiologic evidence thus implicates hypokalemia or reduced K^+ intake in the pathogenesis of hypertension, heart failure, and stroke.[1] Hypokalemia also causes a host of structural and functional changes in the kidney, whereas hyperkalemia in turn has a significant effect on the ability to excrete an acid urine due to interference with the urinary excretion of ammonium (NH_4^+).

Potassium is almost exclusively an intracellular cation, with only 2% of total body K^+ contained within the extracellular fluid. Extracellular K^+ is maintained within a very narrow range by three major mechanisms. First, the distribution of K^+ between the intracellular and extracellular space is determined by the activity of a number of widely expressed and/or ubiquitous transport pathways. A net increase in cellular uptake can thus cause transient hypokalemia, whereas impairment of cellular uptake can lead to hyperkalemia. Second, the colon has the ability to absorb and secrete K^+, with significant mechanistic and regulatory similarities to renal K^+ transport. However, the colon has a relatively limited capacity for K^+ excretion, and a third mechanism, changes in renal K^+ excretion, plays the dominant role in responding to changes in K^+ intake. Regulated increases in K^+ secretion by the connecting tubule (CNT) and the cortical collecting duct (CCD) play a critical role in the response to hyperkalemia and K^+ loading, whereas inhibition of K^+ secretion and increases in the reabsorption of K^+ by the CCD and the outer medullary collecting duct (OMCD) function in the response to hypokalemia or K^+ deprivation.

This chapter reviews the renal and extrarenal mechanisms of K^+ homeostasis, with primary emphasis on the physiology of renal K^+ transport.

Extrarenal Potassium Homeostasis

The intracellular accumulation of K^+ against its electrochemical gradient is an energy-consuming process, mediated by the ubiquitous Na^+/K^+–ATPase. The Na^+/K^+–ATPase functions as an electrogenic pump, with a transport stoichiometry of three intracellular Na^+ ions to two extracellular K^+ ions. The enzyme complex is made up of a tissue-specific combination of multiple α, β, and γ subunits, which are further subject to tissue-specific patterns of regulation. Cardiac glycosides (i.e., digoxin and ouabain) bind to the α subunits of Na^+/K^+–ATPase at an exposed extracellular hairpin loop that also contains the major binding sites for extracellular K^+.[2] The binding of digoxin and K^+ to the Na^+/K^+–ATPase complex is thus mutually antagonistic, explaining in part the potentiation of digoxin toxicity by hypokalemia.[3] Although the four α subunits have equivalent affinity for ouabain, they differ significantly in the intrinsic K^+/ouabain antagonism.[4] Ouabain binding to isozymes containing the ubiquitous α1 subunit is relatively insensitive to K^+ concentrations within the physiologic range, such that this isozyme is protected from digoxin under conditions wherein cardiac α2 and α3 subunits, the therapeutic targets of digoxin, are inhibited.[4] Notably, the digoxin/ouabain binding site of α subunits is highly conserved, suggesting a potential role in the physiologic response to endogenous ouabain/digoxinlike compounds. Consistent with this hypothesis, a mouse strain that expresses α2 subunits with engineered resistance to ouabain is strikingly resistant to ouabain-induced hypertension and to adrenocorticotrophic hormone-dependent hypertension,[5] the latter of which is known to involve an increase in circulating ouabainlike glycosides.

Potassium can also accumulate in cells by coupling to the gradient for Na^+ entry, entering via the electroneutral Na^+-K^+-$2Cl^-$ cotransporters NKCC1 and NKCC2. The NKCC2 protein is found only at the apical membrane of

the thick ascending limb (TAL) and the macula densa cells, where it functions in transepithelial salt transport and tubular regulation of renin release (see Potassium Transport in the Thick Ascending Limb).[6] NKCC1 is widely expressed in multiple tissues,[6] including muscle, where it modulates extrarenal K^+ homeostasis.[7] The cotransport of K^+-Cl^- by the four K^+-Cl^- cotransporters (KCC1 to 4) can also function in the transfer of K^+ across membranes; although the KCCs typically function as efflux pathways, they can mediate influx when extracellular K^+ increases.[6]

Skeletal muscle contains approximately 75% of the body's potassium and exerts considerable influence on extracellular K^+. Skeletal muscle Na^+/K^+–ATPase activity in particular is a major determinant of the capacity for extrarenal K^+ homeostasis. Hypokalemia induces a marked decrease in muscle K^+ content and Na^+/K^+–ATPase activity, an altruistic[8] mechanism to regulate plasma K^+. This adaptation is primarily mediated by dramatic decreases in the protein abundance of the α-2 subunit of Na^+/K^+–ATPase. In contrast, hyperkalemia due to potassium loading is associated with adaptive increases in muscle K^+ content and Na^+/K^+–ATPase activity.[9] These interactions are reflected in the relationship between physical activity and the ability to regulate extracellular K^+ during exercise[10]; exercise training is associated with increases in muscle Na^+/K^+–ATPase concentration and activity, with reduced interstitial K^+ in trained muscles and an enhanced recovery of plasma K^+ after defined amounts of exercise.[10]

Several hormones have been implicated in the control of extrarenal K^+ homeostasis. In particular, increases in plasma K^+ have a stimulatory effect on insulin levels,[11] which in turn stimulates the uptake of K^+ by muscles, the liver, and other tissues. In contrast, insulin-stimulated K^+ uptake is rapidly reduced by 2 days of K^+ depletion, prior to a modest drop in plasma K^+,[12] and in the absence of a change in plasma K^+ in rats subject to a lesser K^+ restriction for 14 days.[13] Insulin activates muscle Na^+/K^+–ATPase, inducing the translocation of the Na^+/K^+–ATPase α-2 subunit to the plasma membrane with a lesser effect on the α1 subunit.[14] This translocation is dependent on the activity of phosphoinositide-3 (PI-3) kinase,[14] which itself also binds to a proline-rich motif in the N-terminus of the α subunit; the activation of PI3-kinase by insulin induces phosphatase enzymes to dephosphorylate a specific serine residue adjacent to the PI3-kinase binding domain. Trafficking of Na^+/K^+–ATPase to the cell surface also requires phosphorylation of an adjacent tyrosine residue, perhaps catalyzed by the tyrosine kinase activity of the insulin receptor itself.[15] In addition, the serum and glucocorticoid-induced kinase 1 (SGK1) plays a critical role in insulin-stimulated K^+ uptake via stimulatory effects on Na^+/K^+–ATPase activity and/or the Na^+-K^+–$2Cl^-$ cotransport.[16] The hypokalemic effect of insulin plus glucose is thus blunted in SGK1 knockout mice, with a marked reduction in hepatic insulin-stimulated K^+ uptake.[16]

The sympathetic nervous system also affects the balance between extracellular and intracellular K^+. The uptake of K^+ by the liver and muscles is stimulated via β_2 receptors. This hypokalemic effect of catecholamines is independent of changes in circulating insulin and has been reported in nephrectomized animals.[17] The cellular mechanisms whereby catecholamines induce K^+ uptake in muscles include an activation of the Na^+/K^+–ATPase, likely via increases in cyclic-AMP (cAMP).[18] Skeletal muscle β-adrenergic receptors also activate NKCC1, which may account for as much as one third of the uptake response.[7] In contrast to β-adrenergic stimulation, α-adrenergic agonists impair the ability to buffer increases in K^+ induced via intravenous loading or by exercise[19]; the transport mechanisms whereby this occurs are not known. β-adrenergic stimulation increases K^+ uptake during exercise to lessen exercise-induced hyperkalemia, whereas α-adrenergic mechanisms help blunt the ensuing postexercise nadir.[19]

The efflux of K^+ out of cells is primarily mediated by K^+ channels, which comprise the largest group of ion channels in the human genome. There are three major subclasses of mammalian K^+ channels: the six-transmembrane domain (TMD) family, which encompasses both the voltage-sensitive and Ca^{2+}-activated K^+ channels; the two-pore, four TMD family; and the two TMD family of inward rectifying K^+ (Kir) channels. There is tremendous genomic variety in human K^+ channels. Further complexity is generated by the presence of multiple accessory subunits and alternative patterns of mRNA splicing. Not surprisingly, an increasing number and variety of K^+ channels have been implicated in the control of K^+ homeostasis and the membrane potential of excitable cells such as muscle and heart, with important, evolving roles in the pathophysiology of potassium disorders. Adrenal K^+ channels have also been implicated in the adrenal release of aldosterone induced by hyperkalemia.[20,21] The emphasis in this chapter is on renal K^+ channels, particularly those in the distal nephron that mediate K^+ secretion.

Potassium Transport in the Proximal Tubule

The proximal tubule reabsorbs 50% to 70% of filtered K^+ (Fig. 6.1). Proximal tubules generate minimal transepithelial K^+ gradients, and the fractional reabsorption of K^+ by this nephron segment is similar to that of Na^+.[22] K^+ absorption thus follows that of fluid, Na^+, and other solutes,[23] such that proximal tubules do not play a direct role in regulated renal K^+ excretion. However, changes in Na^+-Cl^- reabsorption by the proximal tubule have considerable effects on distal tubular flow and distal tubular Na^+ delivery, with attendant effects on the excretory capacity for K^+. In addition, K^+ loading has significant effects on proximal tubular Na^+-Cl^- reabsorption. Thus, the intravenous infusion of K^+-Cl^- or increases in the peritubular K^+ concentration causes an inhibition of proximal tubular Na^+-Cl^- reabsorption[24]; this serves to increase both distal tubular flow and distal tubular Na^+ delivery, thus increasing distal K^+ secretion after K^+ loading.

The mechanisms involved in transepithelial K^+ transport by the proximal tubule are not completely clear, although

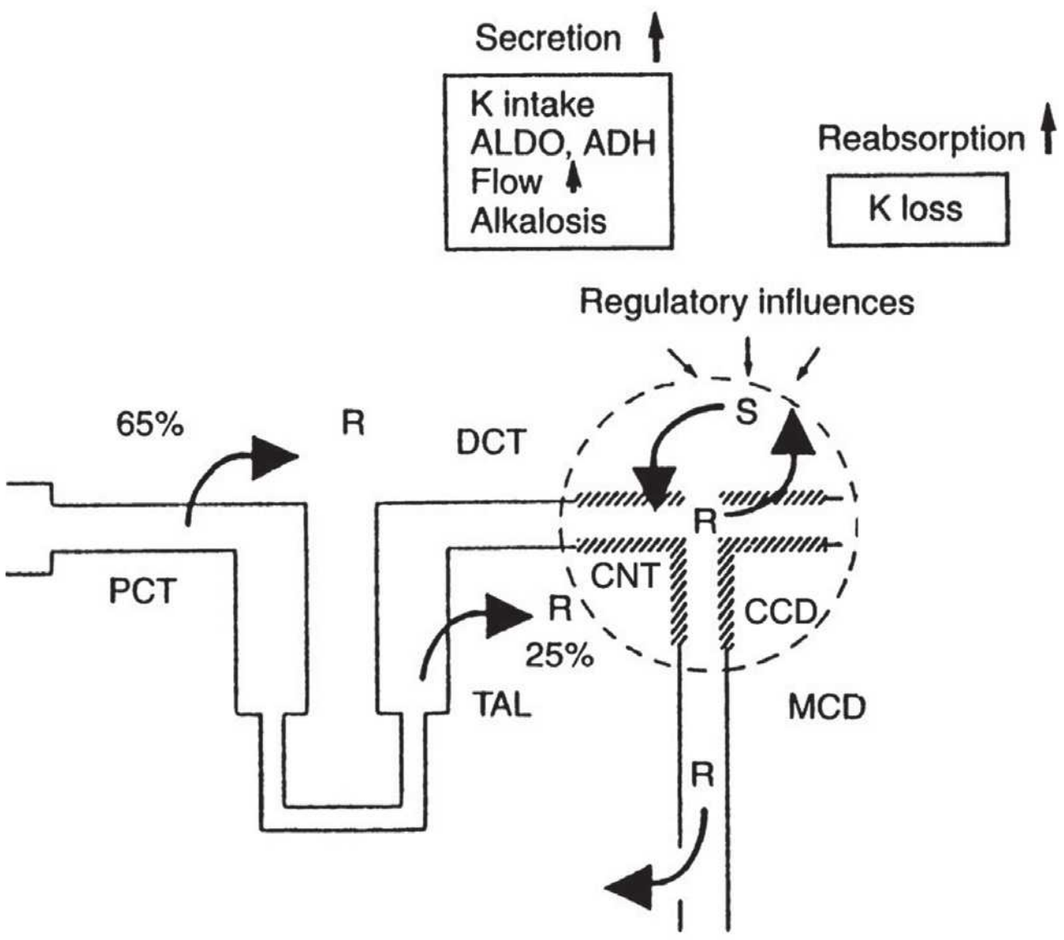

FIGURE 6.1 Potassium transport along the nephron. Approximately 90% of filtered K^+ is reabsorbed by the proximal tubule and the loop of Henle. K^+ is secreted in the connecting tubule and the cortical collecting duct; net reabsorption occurs in response to K^+ depletion, primarily within the medullary collecting duct. *PCT*, proximal tubule; *TAL*, thick ascending limb; *DCT*, distal convoluted tubule; *CNT*, connecting tubule; *CCD*, cortical collecting duct; *S*, secretion; *R*, reabsorption; *ALDO*, aldosterone; *ADH*, antidiuretic hormone; *MCD*, medullary collecting duct.

active transport does not appear to play a major role.[23,25] Luminal barium has modest effects on transepithelial K^+ transport, suggesting a component of transcellular transport via barium-sensitive K^+ channels.[26] However, the bulk of K^+ transport is thought to occur via the paracellular pathway,[26,27] driven by the lumen-positive potential difference in the mid-to-late proximal tubule. The total K^+ permeability of the proximal tubule is thus quite high due to the high permeability of the paracellular pathway.[26, 27] The combination of luminal K^+ concentrations that are ~10% higher than that of plasma, a lumen-positive potential difference of ~2 mV, and high paracellular permeability leads to considerable paracellular absorption in the proximal tubule. The tight junction protein claudin-2 plays a key role in paracellular cation transport by the proximal tubule; targeted deletion in knockout mice generates a "tight" epithelium in the proximal tubule, with a reduction in Na^+, Cl^-, and fluid absorption.[28] The loss of claudin-2 expression does not affect the ultrastructure of tight junctions, but does lead to a reduction in paracellular cation permeability.[28]

The absorption of K^+ across the paracellular pathway is thought to occur via convective transport—solvent drag due to frictional interactions between water and K^+—rather than diffusional transport.[29] Notably, however, the primary pathway for water movement in the proximal tubule is quite conclusively transcellular, via water channels in the apical and basolateral membrane. Therefore, the apparent convective transport of K^+ would have to constitute pseudosolvent drag, with uncharacterized, coordinating interactions between water traversing the transcellular route and the diffusion of K^+ along the claudin-dependent paracellular pathway.[29]

The Loop of Henle and Medullary Potassium Recycling

Transport by the loop of Henle functions in medullary K^+ recycling (Fig. 6.2). A considerable fraction of K^+ secreted by the CCD is reabsorbed by the medullary collecting ducts and is then secreted into the late proximal tubule and/or the descending thin limbs of long-looped nephrons.[30] In potassium-loaded rats there is thus a doubling of luminal K^+ in terminal thin descending limbs, with a sharp drop after the inhibition of CCD K^+ secretion by amiloride.[31] Enhancement of CCD K^+ secretion with the treatment of 1-desamino-8-D-arginine vasopressin (dDAVP) also results in an increase in luminal K^+ in descending thin limbs.[32] This recycling pathway (i.e., secretion in CCD, absorption in the OMCD and the inner medullary collecting duct (IMCD), secretion in the descending thin limb)

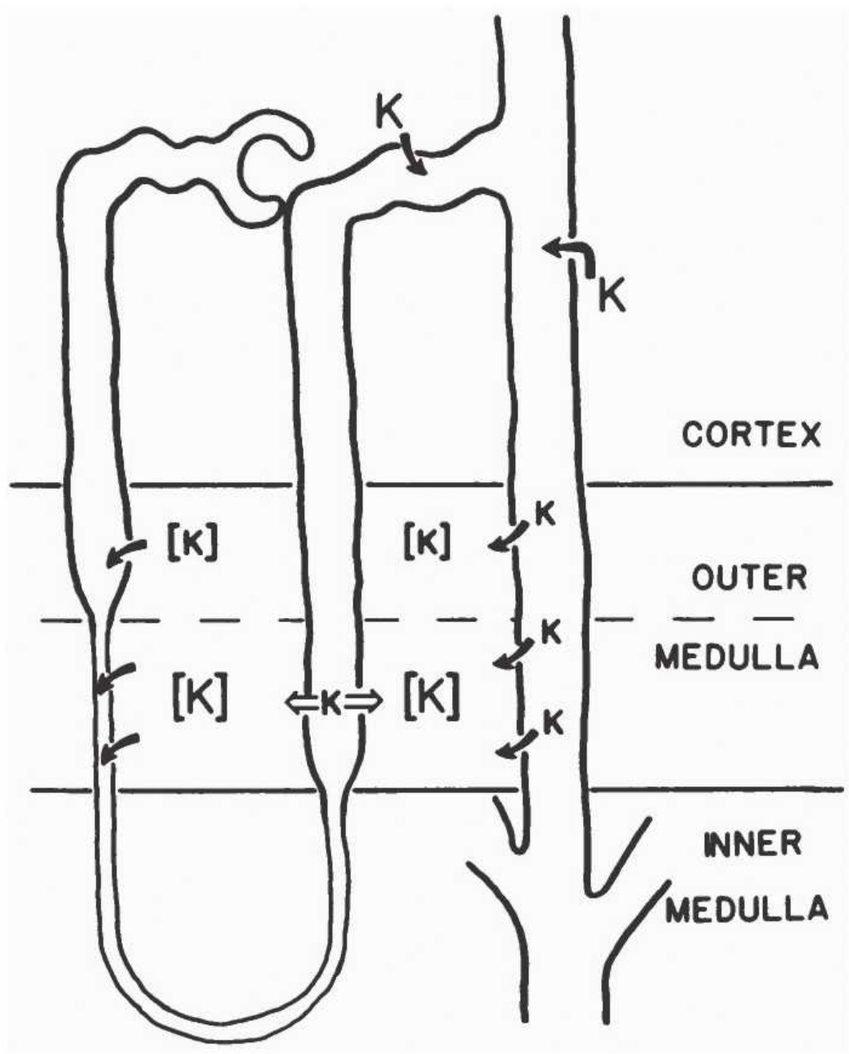

FIGURE 6.2 A schematic representation of medullary K^+ recycling. Medullary interstitial K^+ increases considerably after dietary K^+ loading due to the combined effects of secretion in the cortical collecting duct (CCD), absorption in the outer medullary collecting duct (OMCD), the thick ascending limb (TAL), and the inner medullary collecting duct (IMCD), and secretion in the descending thin limb (see text for details). (From Stokes JB. Consequences of potassium recycling in the renal medulla. Effects of ion transport by the medullary thick ascending limb of Henle's loop. *J Clin Invest*. 1982;70:219–229.)

is associated with a marked increase in medullary interstitial K^+ concentration. Passive transepithelial K^+ absorption by the thin ascending limb and active absorption by the thick ascending limb (TAL)[33] also contribute to this increase in interstitial K^+ (Fig. 6.2). Specifically, the absorption of K^+ by the ascending thin limb, TAL, and OMCD exceeds the secretion by descending thin limbs, thus concentrating K^+ within the interstitium.

K^+ is secreted into descending thin limbs by passive diffusion, driven by the high medullary interstitial K^+ concentration. Descending thin limbs thus have a very high K^+ permeability, without evidence for active transepithelial K^+ transport.[34] Transepithelial K^+ transport by ascending thin limbs has not been measured; however, as is the case for Na^+-Cl^- transport,[35] the absorption of K^+ by thin ascending limbs is presumably passive, via the paracellular pathway.

The physiologic significance of medullary K^+ recycling is not completely clear. However, an increase in interstitial K^+ from 5 to 25 mM dramatically inhibits Cl^- transport by perfused thick ascending limbs.[33] By inhibiting Na^+-Cl^- absorption by the TAL, increases in interstitial K^+ would increase Na^+ delivery to the CNT and CCD, thus enhancing the lumen-negative potential difference (PD) in these tubules and increasing K^+ secretion.[33] A high K^+ diet also reduces the absorption of K^+ by the TAL[36] (i.e., there are direct effects on K^+ secretion by the TAL). The marked increase in medullary interstitial K^+ after dietary K^+ loading is also postulated to limit the difference between luminal and peritubular K^+ in the collecting duct, thus minimizing passive K^+ loss from the collecting duct.

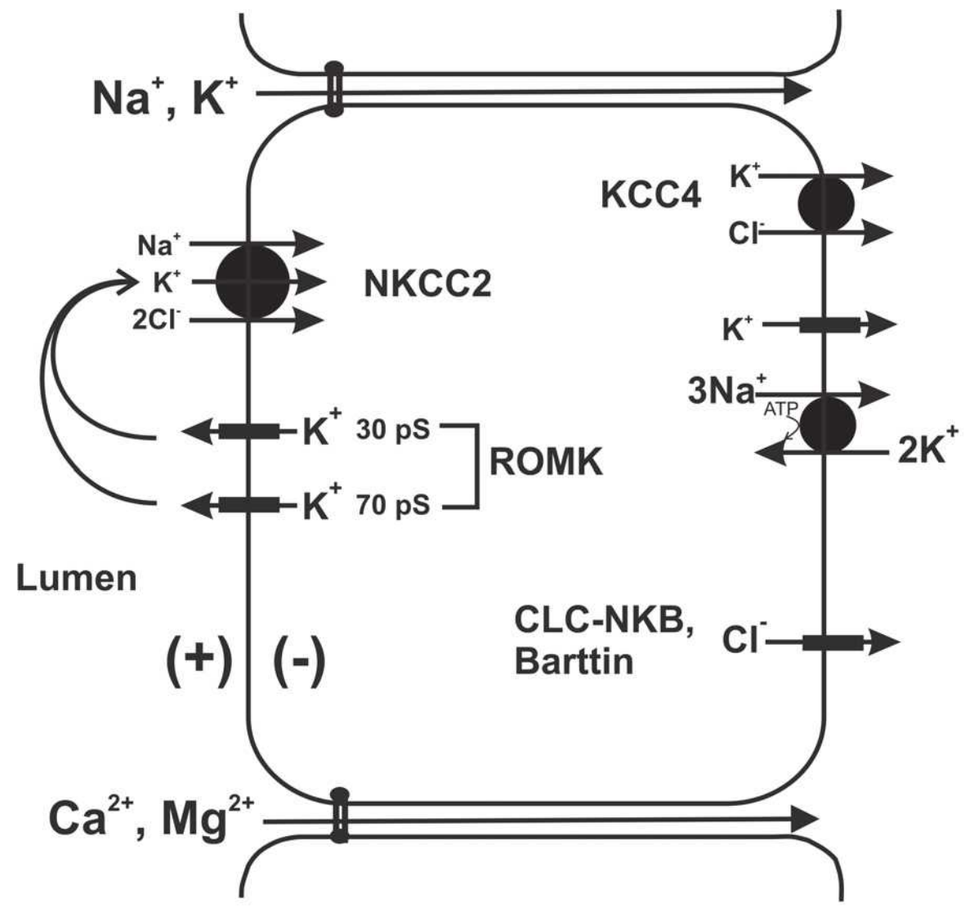

FIGURE 6.3 The potassium transport pathways in the thick ascending limb (TAL); see text for details. *NKCC2*, Na^+-K^+-2Cl– cotransporter 2; *ROMK*, renal outer medullary K^+ channel; *CLC-NKB*, human Cl– channel; *Barttin*, Cl– channel subunit; *KCC4*, K^+-Cl– cotransporter 4.

Potassium Transport in the Thick Ascending Limb

Active transepithelial K^+ transport across the TAL includes both a transcellular component, via the apical Na^+-K^+-$2Cl^-$ cotransporter NKCC2, and a paracellular pathway (Fig. 6.3). Potassium transport appears to differ in the major morphologic subtypes of TAL cells (i.e., rough- and smooth-surfaced cells). Morphologically, the TAL begins abruptly after the thin ascending limb of long-looped nephrons and after the aquaporin-negative segment of short-limbed nephrons. The TAL then extends into the renal cortex, where it meets its parent glomerulus at the vascular pole; the plaque of cells at this junction forms the macula densa, which functions as the tubular sensor for both tubuloglomerular feedback and the tubular regulation of renin release by the juxtaglomerular apparatus. Cells in the medullary TAL are 7 to 8 μm in height, with extensive invaginations of the basolateral plasma membrane and interdigitations between adjacent cells[37]; cells in the cortical TAL are considerably shorter, about 2 μm in height at the end of the cortical TAL in rabbits, with less mitochondria and a simpler basolateral membrane.[37] Macula densa cells also lack the lateral cell processes and interdigitations that are characteristic of medullary TAL cells.[37]

Scanning electron microscopy has revealed that the TAL of both rats[38] and hamsters[39] contains two morphologic subtypes, a rough-surfaced cell type (R cells) with prominent apical microvilli and a smooth-surfaced cell type (S cells) with an abundance of subapical vesicles.[37,40] In the hamster TAL, cells can also be separated into those with high apical and low basolateral K^+ conductance and a low basolateral Cl^- conductance (LBC cells), versus a second population with low apical and high basolateral K^+ conductance, combined with high basolateral Cl^- conductance (HBC) cells.[39,41] The relative frequency of the morphologic and functional subtypes in the cortical and medullary TAL suggests that HBC cells correspond to S cells and LBC cells correspond to R cells.[39]

Morphologic and functional heterogeneity notwithstanding, the cells of the medullary TAL, the cortical TAL, and the macula densa are presumed to share the same basic or at least composite transport mechanisms (see Fig. 6.3). Na^+-Cl^- reabsorption by the TAL is a secondarily active process, driven by the favorable electrochemical gradient for Na^+ established by the basolateral Na^+/K^+–ATPase.[42] The Na^+, K^+, and Cl^- ions are cotransported across by the apical membrane by an electroneutral Na^+-K^+-$2Cl^-$ cotransporter; this transporter generally requires the simultaneous presence of all three ions, such that the transport of Na^+ and Cl^- across the epithelium is mutually codependent and dependent on the luminal presence of K^+. An apical Na^+-K^+-$2Cl^-$ cotransport is mediated by the cation–chloride cotransporter NKCC2, encoded by the SLC12A1 gene.[6] Functional expression of NKCC2 in Xenopus laevis oocytes yields Cl^--dependent and Na^+-dependent uptake of $^{86}Rb^+$ (a radioactive substitute for K^+) and Cl^--dependent and K^+-dependent uptake of $^{22}Na^+$.[6] NKCC2 is sensitive to

micromolar concentrations of furosemide, bumetanide, and other loop diuretics.[6]

Immunofluorescence indicates the expression of the NKCC2 protein along the entire length of the TAL.[6] In particular, immunoelectron microscopy reveals the expression in both rough (R; see previous) and smooth (S) cells of the TAL (see previous).[40] NKCC2 expression in subapical vesicles is particularly prominent in smooth cells,[40] suggesting a role for vesicular trafficking in the regulation of NKCC2. NKCC2 is also expressed in macula densa cells,[40] which have been shown to possess apical Na^+-K^+-$2Cl^-$ cotransport activity. Both tubuloglomerular feedback (TGF) and renal renin secretion are controlled by NKCC2 in the macula densa; luminal loop diuretics block both TGF and the suppression of renin release by the luminal Cl^-.[6]

Microperfused TALs develop a lumen-positive PD during perfusion with Na^+-Cl^-.[43] This lumen-positive PD plays a critical role in the physiology of the TAL, driving the paracellular transport of Na^+, K^+, Ca^{2+}, and Mg^{2+} (see Fig. 6.3). Originally attributed to electrogenic Cl^- transport,[43] the lumen-positive, transepithelial PD in the TAL is generated by the combination of apical K^+ channels and basolateral Cl^- channels.[42] The conductivity of the apical membrane of TAL cells is predominantly, if not exclusively, K^+ selective. Luminal recycling of K^+ via Na^+-K^+-$2Cl^-$ cotransport and apical K^+ channels, along with basolateral depolarization due to Cl^- exit through Cl^- channels, results in the lumen-positive transepithelial PD.[42]

Several lines of evidence indicate that apical K^+ channels are required for transepithelial Na^+-Cl^- transport by the TAL.[42] First, the removal of K^+ from luminal perfusates results in a marked decrease in Na^+-Cl^- reabsorption by the TAL, as measured by a short circuit current; the residual Na^+-Cl^- transport in the absence of luminal K^+ is sustained by the exit of K^+ via apical K^+ channels, because the combination of K^+ removal and a luminal K^+ channel inhibitor (barium) almost abolishes the short-circuit current.[44] Apical K^+ channels are thus required for the activity of NKCC2, the apical Na^+-K^+-$2Cl^-$ cotransporter; the low luminal concentration of K^+ in this nephron segment would otherwise become limiting for transepithelial Na^+-Cl^- transport. Second, the net transport of K^+ across perfused TAL is $<$10% that of Na^+ and Cl^-[33]; ~90% of the K^+ transported by NKCC2 is recycled across the apical membrane via K^+ channels, resulting in minimal net K^+ absorption by the TAL.[42] Third, the intracellular K^+ activity of perfused TAL cells is ~15 to 20 mV above equilibrium, due to furosemide-sensitive entry of K^+ via NKCC2.[45] Given an estimated apical K^+ conductivity of ~12 m per square centimeter, this intracellular K^+ activity yields a calculated K^+ current of ~200 μA per square centimeter; this corresponds quantitatively to the uptake of K^+ by the apical Na^+-K^+-$2Cl^-$ cotransporter. Finally, the observation that Bartter syndrome can be caused by mutations in ROMK (renal outer medullary K^+ channel),[46] a critical component of apical K^+ channels in the TAL (see the following), provides genetic proof for the importance of K^+ channels in Na^+-Cl^- absorption by the TAL. Clearance studies in ROMK-deficient mice also indicate that ~80% of NKCC2 activity is dependent on expression of the ROMK K^+ channel with the TAL.[47]

Three types of apical K^+ channels have been identified in the TAL, a 30 pS channel, a 70 pS channel, and a high-conductance, calcium-activated maxi K^+ channel.[48–50] The higher open probability and greater density of the 30 pS and 70 pS channels, versus the maxi K^+ channel, suggest that these are the primary routes for K^+ recycling across the apical membrane; the 70 pS channel in turn appears to mediate ~80% of the apical K^+ conductance of TAL cells.[51] The low conductance 30 pS channel shares several electrophysiologic and regulatory characteristics with ROMK, the cardinal inward-rectifying K^+ channel (KIR 1.1) that was initially cloned from the renal outer medulla.[52,53] ROMK protein has been identified at the apical membrane of the medullary TAL, the cortical TAL, and the macula densa.[54] Furthermore, the 30 pS channel is also absent from the apical membrane of knockout mice with a homozygous deletion of the gene encoding ROMK.[55] Notably, not all cells in the TAL are labeled with anti-ROMK antibodies,[54,56] suggesting that ROMK might be absent in the HBC cells with high basolateral Cl^- conductance and low apical/high basolateral K^+ conductance (see also previous).[39,41] HBC cells are thought to correspond to the smooth-surfaced morphologic subtype of TAL cells (S cells)[39]; however, distribution of the ROMK protein by immunoelectron microscopy has not as yet been published, hence correlation cannot be made as of yet between morphology and ROMK expression.

Alternative splicing and alternative promoter use of the KCNJ1 gene encoding ROMK/KIR1.1 generates three different protein isoforms, differing in the extreme aminoterminal amino acid sequence: ROMK1, ROMK2, and ROMK3.[57] Although the functional significance of this variation is not clear, these isoforms are differentially distributed along the nephron. ROMK1 is exclusive to the CNT, CCD, and OMCD; ROMK2 is expressed from the TAL to the CCD; and ROMK3 is expressed in the TAL and the distal convoluted tubule (DCT).[57] Apical ROMK channels in the TAL are thus a mixture of ROMK2 and ROMK3 proteins.

ROMK clearly plays a critical role in Na^+-Cl^- absorption by the TAL, given that loss-of-function mutations in this gene are associated with Bartter syndrome.[46] The role of ROMK in Bartter syndrome was initially discordant with the data indicating that the 70 pS K^+ channel is the dominant conductance at the apical membrane of TAL cells[51]; heterologous expression of the ROMK protein in *Xenopus* oocytes had yielded a channel with a conductance of ~30 pS, suggesting that the 70 pS channel was distinct from ROMK. This paradox was resolved by the observation that the 70 pS channel is absent from the TAL of ROMK knockout mice, indicating that ROMK proteins form a subunit of the 70 pS channel.[58] ROMK activity in the TAL is clearly modulated by association with other proteins. That coassociation with other subunits generates the 70 pS channel and is perfectly compatible with

the known physiology of this channel protein. ROMK thus associates with scaffolding proteins NHERF-1 and NHERF-2 via the C-terminal PDZ-binding motif of ROMK; NHERF-2 is coexpressed with ROMK in the TAL.[59] The association of ROMK with NHERFs brings ROMK into closer proximity to the cystic fibrosis transmembrane regulator (CFTR) protein.[59] This ROMK-CFTR interaction is in turn required for reconstitution of the native ATP and glibenclamide sensitivity of apical K^+ channels in the TAL.[60]

TAL cells are phenotypically defined by the apical expression of uromodulin or Tamm-Horsfall glycoprotein (THP), a TAL-specific, GPI-linked membrane protein that is shed into the tubular lumen as the most abundant protein in normal human urine. THP interacts with ROMK protein in yeast two-hybrid screens and activates the channel in coexpression experiments.[61] THP also exhibits functional interactions with NKCC2, activating the cotransporter in coexpression experiments.[62] Membrane trafficking of both ROMK and NKCC2 proteins is affected in THP knockout mice, with greater accumulation of both transport proteins in subapical vesicles. THP knockout mice also exhibit a blunted natriuretic and kaliuretic response to furosemide, which is consistent with a partial deficiency in TAL function.[62] Mutations of the THP gene cause the allelic disorders familial juvenile hyperuricemic nephropathy, medullary cystic kidney disease type II, and glomerulocystic kidney disease. All three disorders share reduced expression of THP at the apical membrane with retention in the endoplasmic reticulum. The associated defects in NKCC2 and ROMK may explain the renal salt wasting and hyperuricemia that can be associated with these genetic disorders.[61,62]

Potassium Secretion by the Distal Convoluted Tubule, Connecting Tubule, and Cortical Collecting Duct

Approximately 90% of filtered K^+ is reabsorbed by the proximal tubule and the loop of Henle (Fig. 6.1); the final adjustments in renal K^+ excretion occur in the downstream distal nephron. The bulk of regulated secretion occurs in the CNT and CCD, whereas K^+ reabsorption primarily occurs in the OMCD (see the following). K^+ secretion is initially detectable in the early DCT, wherein cells expressing the thiazide-sensitive Na^+-Cl^- cotransporter (NCC) express ROMK, the apical K^+ secretory channel (see the following).[54] More recent studies in rat kidneys have localized the protein to both DCT1 and DCT2 segments.[56] Classically, the CCD is considered the primary site for distal K^+ secretion, largely due to the greater ease with which this segment can be perfused and studied. However, as in Na^+ absorption (see below),[63,64] the bulk of distal K^+ secretion appears to occur prior to the CCD,[22] within the CNT.[65] In addition to a lesser endowment with absorptive apical epithelial Na^+ channels (ENaC) and secretory K^+ channels, water absorption in the vasopressin-responsive CCD limits K^+ secretion by allowing the concentration of luminal K^+ to rise toward equilibrium.[66] Water permeability of the CNT is considerably lower than that of the CCD,[66] with a significant number of early (CNT1) CNT cells that do not express aquaporin-2 in the absence of vasopressin. Aquaporin-2 expression is also low or undetectable in CNT1 cells on a high K^+ diet,[56] further optimizing these early CNT segments for K^+ secretion.

The apical membrane of CNT cells and principal cells contain prominent Na^+ and K^+ conductances,[63,65,67] without a measurable apical conductance for Cl^-.[68] The entry of Na^+ occurs via the highly selective ENaC, which is sensitive to micromolar concentrations of amiloride (Fig. 6.4). This selective absorption of a positive charge generates a lumen-negative PD, the magnitude of which varies considerably as a function of mineralocorticoid status and other factors. This lumen-negative PD serves to drive the following critical processes: (1) K^+ secretion via apical K^+ channels, (2) paracellular Cl^- transport through the adjacent tight junctions, and (3) electrogenic H^+ secretion via adjacent type A intercalated cells.

The three ENaC subunits are detectable at the apical membrane of CNT cells and principal cells within the CCD, OMCD, and IMCD. Notably, however, several lines of evidence support the hypothesis that the CNT makes the

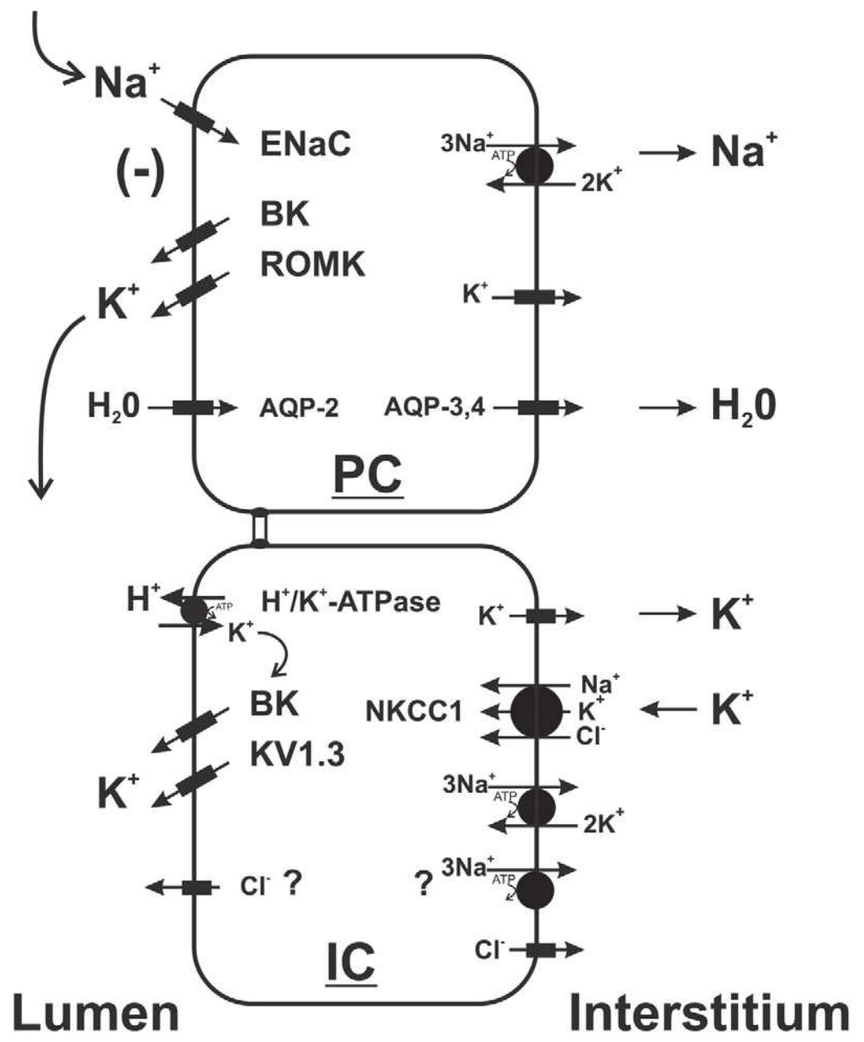

FIGURE 6.4 The transport pathways for potassium in principal/connecting tubule (CNT) cells and intercalated cells. Potassium secretion occurs in CNT and principal cells, driven by the lumen-negative potential difference generated by the epithelial Na^+ channel (ENaC). In intercalated cells, potassium reabsorbed by apical H^+/K^+-ATPase recycles across the apical membrane via K^+ channels; alternatively, in the setting of hypokalemia or potassium deprivation, it exits the cell via basolateral K^+ channels. Potassium that is secreted across intercalated cells enters at the basolateral membrane via NKCC1 and exits via apical BK and Kv1.3 K^+ channels. *BK*, BK K^+ channel; *ROMK*, renal outer medullary K^+ channel; *Kv1.3*, Kv1.3 K^+ channel; *NKCC1*, Na^+-K^+-$2Cl^-$ cotransporter 1.

dominant contribution to amiloride-sensitive Na^+ reabsorption by the distal nephron. First, amiloride-sensitive Na^+ currents in the CNT are two- to fourfold higher than in the CCD; the maximal capacity of the CNT for Na^+ reabsorption is estimated to be ~10 times higher than that of the CCD.[63] Second, targeted deletion of α-ENaC in the collecting duct abolishes amiloride-sensitive currents in CCD principal cells, but does not affect Na^+ or K^+ homeostasis. Thus, the residual ENaC expression in the late DCT and CNT of these knockout mice easily compensates for the loss of the channel in CCD.[69] In contrast, the more extensive deletion of α-ENaC in all aquaporin-2–positive cells in both CNT and CCD causes a profound impairment in Na^+ and K^+ homeostasis.[64] Third, Na^+/K^+–ATPase activity in the CCD is considerably less than that of the DCT[70]; this speaks to a greater capacity for transepithelial Na^+-Cl^- absorption by the DCT and CNT. Fourth, the apical recruitment of ENaC subunits in response to dietary Na^+ restriction begins in the CNT, with progressive recruitment of subunits in the downstream CCD at lower levels of dietary Na^+[71]; under conditions of high Na^+-Cl^- and low K^+ intake, the bulk of aldosterone-stimulated Na^+ transport likely occurs prior to the entry of tubular fluid into the CCD.

In principal cells and CNT cells, the lumen-negative potential difference generated by Na^+ entry via ENaC drives passive K^+ exit through apical K^+ channels. Although there is evolving evidence for ENaC− and Na^+-independent secretion (see also the following),[72] distal K^+ secretion is critically dependent on delivery of adequate luminal Na^+ to the CNT and CCD,[73] essentially ceasing when luminal Na^+ drops below 8 mmol/L.[74] Dietary Na^+ intake also influences K^+ excretion, such that excretion is enhanced by excess Na^+ intake and reduced by Na^+ restriction.[73] Secreted K^+ enters principal cells via the basolateral Na^+/K^+–ATPase, which also generates the gradient that drives apical Na^+ entry via ENaC (Fig. 6.4).

Two major subtypes of apical K^+ channels function in secretion by the CNT and CCD, +/– DCT; a small-conductance (SK) 30 pS channel[55,65] and a large-conductance, Ca^{2+}-activated 150 pS ("maxi-K" or BK) channel.[65,75] The density and high open probability of the SK channel indicates that this pathway alone is sufficient to mediate the bulk of K^+ secretion in the CCD under baseline conditions,[76] hence its designation as the "secretory" K^+ channel. Notably, SK channel density is considerably higher in the CNT than in the CCD,[65] consistent with the greater capacity for Na^+ absorption[63] and K^+ secretion in the CNT. The characteristics of the SK channel are similar to those of the ROMK K^+ channel,[53] and ROMK protein has been localized at the apical membrane of principal cells.[54] SK channel activity is absent from apical membranes of the CCD in homozygous ROMK knockout mice, definitive proof that ROMK is the SK channel.[55] The observation that these knockout mice are normokalemic with an increased excretion of K^+ illustrates the considerable redundancy in distal K^+ secretory pathways[55]; distal K^+ secretion in these mice is mediated by apical BK channels (see the following).[77] Loss-of-function mutations in human KCNJ1 are associated with Bartter syndrome; ROMK expression is critical for the 30 pS and 70 pS channels that generate the lumen-positive PD in the TAL (see Fig. 6.3).[55,58] These patients typically have slightly higher serum K^+ than the other genetic forms of Bartter syndrome,[46] and affected patients with severe neonatal hyperkalemia have also been described; this neonatal hyperkalemia is presumably the result of a transient developmental deficit in apical BK channel activity (see the following).

The apical Ca^{2+}-activated BK channel plays a critical role in *flow*-dependent K^+ secretion by the CNT and CCD.[75] BK channels have a heteromeric structure, with α-subunits that form the ion channel pore and modulatory β-subunits that affect the biophysical, regulatory, and pharmacologic characteristics of the channel complex.[75] BK α-subunit transcripts are expressed in multiple nephron segments, and channel protein is detectable at the apical membrane of principal and intercalated cells in the CCD and CNT.[75] The β subunits are differentially expressed within the distal nephron. Thus β1 subunits are restricted to the CNT,[75] with no expression in intercalated cells,[78] whereas β4 subunits are detectable at the apical membranes of TAL, DCT, and intercalated cells.[78] Increased distal flow has a well-established stimulatory effect on K^+ secretion, due in part to both the enhanced delivery and absorption of Na^+ and to the increased removal of secreted K^+.[73] The pharmacology of flow-dependent K^+ secretion in the CCD is consistent with BK channels,[79] and flow-dependent K^+ secretion is reduced in mice with targeted deletion of the α1 and β1 subunits.[75,80,81] Both mice strains develop hyperaldosteronism that is exacerbated by high-K^+ diet,[81] leading to hypertension in the α1 subunit knockout.[81]

One enigma has been the greater density of BK channels in intercalated cells, in both the CCD[82] and the CNT.[83] This has suggested a major role for intercalated cells in K^+ secretion; however, the much lower density of Na^+/K^+–ATPase activity in intercalated cells has been considered inadequate to support K^+ secretion across the apical membrane.[84] More recent evidence reveals a major role for the basolateral Na^+-K^+-$2Cl^-$ cotransporter NKCC1 in K^+ secretion mediated by apical BK channels (see also Fig. 6.4)[85]; NKCC1 is expressed almost exclusively at the basolateral membrane of intercalated cells,[86] providing an alternative entry pathway for basolateral K^+ secreted at the apical membrane. This still begs the question of how basolateral Na^+ recycles across the basolateral membrane, in the absence of significant Na^+/K^+–ATPase activity; one possibility is an alternative basolateral Na^+ pump, the ouabain-insensitive furosemide-sensitive Na^+–ATPase, a transport activity that has been detected in cell culture models of intercalated cells.[85] At the apical membrane, BK-mediated K^+ secretion is only partially dependent on luminal Na^+[87]; K^+ secretion would eventually hyperpolarize the membrane in the absence of apical Na^+ entry, which is mediated by ENaC in principal cells. An intriguing possibility is that apical Cl^- channels[88] allow for a parallel secretion of K^+ and Cl^- in intercalated cells (see Fig. 6.4).

BK channels also play a critical role in cell volume regulation by intercalated cells, with indirect, flow-mediated influences on distal K^+ secretion. MDCK-C11 cells have an intercalated cell phenotype and express BK α and β4 subunits, as do intercalated cells[78]; shear stress activates BK channels in these cells, leading to a loss of K^+ and cell shrinkage.[89] Mice with a targeted deletion of the β4 subunit exhibit normal K^+ excretion on a normal diet.[84] However, when fed a high K^+ diet, which increases urinary and tubular flow rates and tubular shear stress, the β4-knockout mice develop hyperkalemia with a blunted increase in both K^+ excretion and urinary flow rates. Intercalated cells from β4 knockout may fail to significantly decrease cell volume in response to a high K^+ diet. Intercalated cells thus function as "speed bumps" that protrude into the lumen of distal tubules; flow-activated BK channels reduce the cell volume of intercalated cells after K^+ loading, reducing tubular resistance, increasing tubular flow rates, and increasing distal K^+ secretion.[84]

The physiologic rationale for the presence of two apical secretory K^+ channels—ROMK/SK and BK channels—is not completely clear. However, the high density and higher open probability of SK/ROMK channels is perhaps better suited for a role in basal K^+ secretion, with additional recruitment of the higher capacity, flow-activated BK channels when additional K^+ secretion is required.[75] The evolving evidence also indicates that BK channels function in partially Na^+-independent K^+ secretion by intercalated cells, with ROMK functioning in ENaC- and Na^+-dependent K^+ excretion by DCT, CNT, and CCD cells. Regardless, at the whole organ level, the two K^+ channels can substitute for one another, with BK-dependent K^+ secretion in ROMK knockout mice[77] and an upregulation of ROMK in the distal nephron of α1 subunit BK knockout mice.[80]

Other K^+ channels reportedly expressed at the luminal membranes of the CNT and CCD include voltage-sensitive channels such as Kv1.3,[90] double-pore K^+ channels such as TWIK-1,[91] and KCNQ1.[92] KCNQ1 mediates K^+ secretion in the inner ear and is expressed at the apical membrane of principal cells in the CCD,[92] whereas TWIK-1 is expressed at the apical membrane of intercalated cells.[91] The roles of these channels in renal K^+ secretion or absorption are not fully characterized. However, Kv1.3 may play a role in distal K^+ secretion by intercalated cells (see Fig. 6.4) in that luminal margatoxin, a specific blocker of this channel, reduces K^+ secretion in CCDs of rat kidneys from animals on a high K^+ diet (see also previous).[90] Other apical K^+ channels in the distal nephron subserve other physiologic functions. For example, the apical Kv1.1 channel is critically involved in Mg^{2+} transport by the DCT, likely by hyperpolarizing the apical membrane and increasing the driving force for Mg^{2+} influx via transient receptor potential cation channel 6 (TRPM6); missense mutations in Kv1.1 are a cause of genetic hypomagnesemia.[93]

K^+ channels present at the basolateral membrane of principal cells set the resting potential of the basolateral membrane and function in K^+ secretion and Na^+ absorption at the apical membrane, the latter via K^+ recycling at the basolateral membrane to maintain activity of the Na^+/K^+–ATPase. A variety of different K^+ channels have been described in the electrophysiologic characterization of the basolateral membrane of principal cells, which has a number of technical barriers (reviewed by Gray et al.[94]). However, a single predominant activity can be identified in principal cells from the rat CCD, using whole-cell recording techniques under conditions in which ROMK is inhibited (low intracellular pH or the presence of the ROMK inhibitor tertiapin-Q).[94] This basolateral current is tetraethylammonium (TEA) insensitive, barium sensitive, and acid sensitive (pKa ~6.5), with a conductance of ~17 pS and weak inward rectification. These properties do not correspond exactly to specific characterized K^+ channels, or combinations thereof. However, candidate inward-rectifying K^+ channel subunits that have been localized at the basolateral membrane of the CCD include KIR4.1, KIR5.1, KIR7.1, and KIR2.3.[94] A more recent report suggests that KIR4.1/KIR5.1 channels generate a predominant 40 pS basolateral K^+ channel in murine principal cells.[95] Notably, basolateral K^+ channel activity increases on a high K^+ diet, suggesting a role in transepithelial K^+ secretion.[94]

Similar K^+ channels are found at the basolateral membrane of DCT cells. Cell-attached patches in the basolateral membranes of microdissected DCTs detect an inward rectifying K^+ channel with characteristics similar to heteromeric KIR4.1/KIR5.1 and KIR4.2/KIR5.1 channels.[96] Basolateral membranes of the DCT express immunoreactive KIR4.1[97] and KIR5.1[98] proteins, and DCT cells express KIR4.2 mRNA.[96] Patients with loss-of-function mutations in the KCNJ10 gene that encodes KIR4.1 develop a syndrome of epilepsy, ataxia, sensorineural deafness, and renal tubulopathy.[97,99] The tubulopathy phenotype encompasses hypokalemia, metabolic alkalosis, hypocalciuria, and hypomagnesemia[97,99]; KIR4.1 knockout mice demonstrate a greater natriuresis than littermate controls, in addition to hypocalciuria.[97] As in principal cells, KIR4.1/KIR5.1 and KIR4.2/KIR5.1 channels at the basolateral membrane of DCT cells are hypothesized to function in basolateral K^+ recycling, maintaining adequate Na^+/K^+–ATPase activity for Na^+-Cl^- absorption and other aspects of DCT function.

In addition to apical K^+ channels, considerable evidence implicates apical K^+-Cl^- cotransport (or functionally equivalent pathways)[100] in distal K^+ secretion.[101] In perfused rat distal tubules, a reduction in luminal Cl^- markedly increases K^+ secretion[102]; the replacement of luminal Cl^- with SO_4^- or gluconate has an equivalent stimulatory effect on K^+ secretion. This anion-dependent component of K^+ secretion is not influenced by luminal Ba^{2+},[102] suggesting that it does not involve apical K^+ channel activity. Perfused surface distal tubules are a mixture of the DCT, the connecting segment, and the initial collecting duct; however, Cl^--coupled K^+ secretion is detectable in both the DCT and in the early CNT.[103] In addition, similar pathways are detectable in rabbit CCD, where a decrease in luminal Cl^- from 112 mmol per liter to 5 mmol per liter increases

K^+ secretion by 48%.[104] A reduction in basolateral Cl^- also decreases K^+ secretion without an effect on transepithelial voltage or Na^+ transport, and the direction of K^+ flux can be reversed by a lumen-to-bath Cl^- gradient, resulting in K^+ absorption.[104] In perfused CCDs from rats treated with a mineralocorticoid, vasopressin increases K^+ secretion.[105] Because this increase in K^+ secretion is resistant to luminal Ba^{2+} (2 mmol per liter), vasopressin may stimulate Cl^--dependent K^+ secretion.[106] Recent pharmacologic studies of perfused tubules are consistent with K^+-Cl^- cotransport mediated by the KCCs[101]; however, of the three renal KCCs, only KCC1 is apically expressed in the distal nephron. Other functional possibilities for Cl^--dependent K^+ secretion include the parallel operation of apical H^+-K^+-exchange and Cl^--HCO_3^- exchange in type B intercalated cells[100] and parallel K^+ and Cl^- channels in type A intercalated cells (see Fig. 6.4).[88]

A provocative study by Frindt and Palmer[72] underscores the importance of ENaC-independent K^+ excretion, be it mediated by apical K^+-Cl^- cotransport and/or by other mechanisms (see also Integrated Regulation of Distal Sodium and Potassium Transport). Rats were infused with amiloride via osmotic minipumps, generating urinary concentrations considered sufficient to inhibit >98% of ENaC activity. Whereas amiloride almost abolished K^+ excretion in rats on a normal K^+ intake, acute and long-term high K^+ diets led to an increasing fraction of K^+ excretion that was independent of ENaC activity (~50% after 7 to 9 days on a high K^+ diet).

Potassium Reabsorption by the Collecting Duct

In addition to K^+ secretion, the distal nephron is capable of considerable K^+ reabsorption, primarily during the restriction of dietary K^+.[22,107,108] This reabsorption is accomplished in large part by intercalated cells in the OMCD, via the activity of apical H^+/K^+–ATPase pumps. Under K^+-replete conditions, apical H^+/K^+–ATPase activity recycles K^+ with an apical K^+ channel, without effect on transepithelial K^+ absorption (see Figure 6.4). Under K^+-restricted conditions, K^+ absorbed via apical H^+/K^+–ATPase appears to exit intercalated cells via a basolateral K^+ channel, thus achieving the transepithelial transport of K^+.[109]

H^+-K^+–ATPase holoenzymes are members of the P-type family of ion transport ATPases, which also includes subunits of the basolateral Na^+-K^+–ATPase.[110] HKα-1 and HKα-2 are also referred to as the gastric and colonic subunits, respectively. A specific HKβ subunit interacts with the HKα subunits to ensure delivery to the cell surface and a complete expression of H^+-K^+–ATPase activity; HKα-2 subunits are also capable of interaction with Na^+-K^+–ATPase β subunits. The pharmacology of H^+-K^+–ATPase holoenzymes differs considerably, such that the gastric HKα-1 is classically sensitive to the H^+-K^+–ATPase inhibitors SCH-28080 and omeprazole and is resistant to ouabain; the colonic HKα-2 subunit is usually sensitive to ouabain and resistant to SCH-28080.[110] Within the kidney, the HKα-1 subunit is expressed at the apical membrane of at least a subset of type A intercalated cells in the distal nephron.[111] HKα-2 distribution in the distal nephron is more diffuse, with robust expression at the apical membrane of type A and B intercalated cells and connecting segment cells, and a lesser expression in principal cells.[112]

HKα-1 and HKα-2 are both constitutively expressed in the distal nephron. However, tubule perfusion of K^+-replete animals suggests a functional dominance of omeprazole/SCH-28080-sensitive, ouabain-resistant H^+-K^+–ATPase activity that is consistent with holoenzymes containing HKα-1.[113] K^+ deprivation increases the overall activity of H^+-K^+–ATPase in the collecting duct, with the emergence of a ouabain-sensitive H^+-K^+–ATPase activity.[114] This is consistent with a relative dominance of HKα-2 during K^+-restricted conditions. Consistent with this pharmacology, K^+-restriction also induces a dramatic upregulation of HKα-2 transcript and protein in the outer and inner medulla during K^+ depletion; HKα-1 expression is unaffected.[115,116] Mice with a targeted deletion of HKα-2 exhibit lower plasma and muscle K^+ than wild-type littermates when maintained on a K^+-deficient diet. However, this appears to be due to a marked loss of K^+ in the colon rather than the kidney, because renal K^+ excretion is appropriately reduced in the K^+-depleted knockout mice.[110] Presumably, the lack of an obvious renal phenotype in either HKα-1[110] or HKα-2[110] knockout mice reflects the marked redundancy in the expression of HKα subunits in the distal nephron. Indeed, collecting ducts from the HKα-1 knockout mice have significant residual ouabain-resistant and SCH-28080-sensitive H^+-K^+–ATPase activities, consistent with the expression of other HKα subunits that confer characteristics similar to the "gastric" H^+-K^+–ATPase.[110] However, data from HKα-1 and HKα-2 knockout mice suggest that compensatory mechanisms in these mice are not accounted for by ATPase-type mechanisms.[117]

The importance of K^+ reabsorption mediated by the collecting duct is dramatically illustrated by the phenotype of transgenic mice with generalized overexpression of a gain-of-function mutation in H^+-K^+–ATPase; this effectively bypasses the redundancy and complexity of this reabsorptive pathway. This mutant HKβ subunit has a tyrosine-to-alanine mutation within the C-terminal tail that abrogates regulated endocytosis from the plasma membrane; these mice have higher plasma K^+ than their wild-type littermates, with approximately half the fractional excretion of K^+.[118]

Finally, it should be noted that considerable evidence implicates the H^+-K^+–ATPases in renal acid secretion.[110] In particular, acid extrusion from intercalated cells is markedly reduced in doubly deficient HKα-1/HKα-2 knockout mice.[110] In addition, the H^+-K^+–ATPases may function directly in Na^+ balance, collaborating, for example, with apical Cl^-:HCO_3^- exchangers in the CCD in apical Na^+-Cl^- uptake.[110]

The Regulation of Distal Tubular Potassium Transport

Modulation of ROMK Activity

ROMK and other KIR channels are inward rectifying (i.e., K^+ flows inward more readily than outward). Even though outward conductance is usually less than inward conductance, K^+ efflux through ROMK predominates in the CNT and CCD because the membrane potential is more positive than the equilibrium potential for K^+. Intracellular magnesium (Mg^{2+})[119] and polyamines[120] play key roles in inward rectification, binding and blocking the pore of the channel from the cytoplasmic side.[121] A single transmembrane residue, asparagine-171 in ROMK1, controls the affinity and the blocking effect of both Mg^{2+} and polyamines.[119,120] Intracellular Mg^{2+} in TAL, DCT, CNT, and principal cells is thought to have a significant effect on ROMK activity, because it inhibits outward ROMK-dependent currents in principal cells.[122] The blocking affinity of Mg^{2+} is enhanced at lower extracellular K^+ concentrations,[122] which should aid in reducing K^+ secretion during hypokalemia and K^+ deficiency. A reduction of this intracellular Mg^{2+} block may also explain the hypokalemia associated with hypomagnesemia, wherein distal K^+ secretion is enhanced.[121]

In addition to inward rectification, the endogenous ROMK channels in the TAL and principal cells exhibit a very high channel open probability (P_o). The high P_o of ROMK is maintained by the combined effects of the binding of phosphatidylinositol-4,5-bisphosphate (PIP_2) to the channel protein, direct channel phosphorylation by protein kinase A (PKA), ATP binding to the ROMK-CFTR complex, and cytoplasmic pH. PIP2 binding to ROMK is thus required to maintain the channel in an open state,[123] whereas cytoplasmic acidification inhibits the channel. PKA phosphorylates ROMK protein at one N-terminal serine and two C-terminal serines[124]: S25, S200, and S294 in the ROMK2 isoform. Phosphorylation of all three sites is required for full channel function. The phosphorylation of the N-terminal site overrides the effect of a carboxy-terminal endoplasmic reticulum retention signal, thus increasing the expression of the channel protein at the cell membrane.[125] The phosphorylation of S200 and S294 maintains the channel in a high P_o state, in part by modulating the effects of PIP_2,[126] ATP,[60] and pH.[127]

Because ROMK channels exhibit such a high P_o, the physiologic regulation of the channel is primarily achieved by regulated changes in the number of active channels on the plasma membrane. The associated mechanisms are discussed in the context of the adaptation to K^+ loading/hyperkalemia and K^+ deprivation/hypokalemia.

Aldosterone and Potassium Secretion

Aldosterone has a potent kaliuretic effect,[128] with important interrelationships between circulating K^+ and aldosterone. Aldosterone release by the adrenal is thus induced by hyperkalemia and/or a high K^+ diet,[129] suggesting an important feedback effect of aldosterone on K^+ homeostasis.[130] Aldosterone also has clinically relevant effects on K^+ homeostasis, with a clear relationship at all levels of serum K^+between circulating levels of the hormone and the ability to excrete K^+.

Renin released from the kidney stimulates aldosterone release from the adrenal via angiotensin-II (AT-II). Renin secretion by juxtaglomerular cells within the afferent arteriole is initiated in response to a signal from the macula densa, specifically a decrease in luminal chloride transported through the Na^+-K^+-$2Cl^-$ cotransporter (NKCC2) at the apical membrane of macula densa cells.[6] In addition to this macula densa signal, decreased renal perfusion pressure and renal sympathetic tone stimulate renal renin secretion.

Hyperkalemia is also an independent and synergistic stimulus for aldosterone release from the adrenal gland,[129] although dietary K^+ loading is less potent than dietary Na^+-Cl^- restriction in increasing circulating aldosterone.[130] The resting membrane potential of adrenal glomerulosa cells is hyperpolarized due to the activity of the "leak" K^+ channels TASK-1 and TASK-3; the combined deletion of genes encoding these channels leads to baseline depolarization of adrenal glomerulosa cells and an increase in plasma aldosterone that is resistant to dietary sodium loading.[20] The KCNJ5 K^+ channel also plays a role, in that mutations that produce a depolarizing acquisition of KCNJ5-mediated Na^+ conductance are associated with adrenal adenomas.[21] AT-II and K^+ both activate Ca^{2+} entry in glomerulosa cells via voltage-sensitive T-type Ca^{2+} channels, primarily Cav3.2.[131] Elevations in extracellular K^+ thus depolarize glomerulosa cells and activate these Ca^{2+} channels, which are independently and synergistically activated by AT-II. The calcium-dependent activation of calcium-calmodulin (CaM)-dependent protein kinase, in turn, activates the synthesis and release of aldosterone via the induction of aldosterone synthase. K^+ and AT-II also enhance the transcription of the Cav3.2 Ca^{2+} channel by abrogating repression of this gene by the neuron restrictive silencing factor (NRS); this ultimately amplifies the induction of aldosterone synthase.[131]

The adrenal release of aldosterone due to increased K^+ is dependent on an intact adrenal renin–angiotensin system, particularly during Na^+-Cl^- restriction. Angiotensin converting enzyme (ACE) inhibitors and angiotensin–receptor blockers (ARBs) completely abrogate the effect of high K^+ on salt-restricted adrenals.[132] Direct, G protein–dependent activation of the TASK-1 and/or TASK-3 K^+ channels by AT_{1A} or AT_{1B} receptors is thought to underlie the effect of AT-II on adrenal aldosterone release,[20] with abrogation of this effect by ARBs or ACE inhibitors. Other clinically relevant activators of adrenal aldosterone release include prostaglandins and catecholamines via increases in cAMP.[133] Finally, atrial natriuretic peptide (ANP) exerts a potent negative effect on aldosterone release induced by K^+ and other stimuli,[134] at least in part by inhibiting early events in aldosterone synthesis.[135] ANP inhibits both renal renin release and adrenal aldosterone release, functions that may be central to the roles of this hormone in the pathophysiology of hyporeninemic hypoaldosteronism.[134]

Within the kidney, aldosterone has no effect on the density of apical SK channels in the CCD[136]; it does however induce a marked increase in the density of apical Na^+ channels in the CNT and CCD.[136] Aldosterone activates ENaC via interrelated effects on the transcription, synthesis, trafficking, and membrane-associated activity of the subunits encoding the channel. Aldosterone is thus induced by a high K^+ diet and strongly stimulates apical ENaC activity, which generates the lumen-negative PD that stimulates K^+ secretion by principal cells. The hormone also has significant effects on the basolateral membrane of principal cells, with dramatic changes in cellular morphology and the length of basolateral membranes.[137] This is accompanied by an increase in basolateral Na^+-K^+–ATPase activity, although it has been difficult to determine how much of these cellular and functional changes are due to enhanced Na^+ entry via apical ENaC. It is, however, known that aldosterone increases the expression of the Na^+-K^+–ATPase α1 and β1 subunits in the CCD[138]; these effects are evidently independent of ENaC activity.[139]

Transcriptional effects of aldosterone also include the induction of the α-ENaC subunit via a glucocorticoid-response element in the promoter of the SCNN1A gene. Aldosterone also relieves a tonic inhibition of the SCNN1A gene by a complex that includes the Dot1a (disruptor of telomere silencing splicing variant a) and the AF9 and AF17 transcription factors.[140] This transcriptional activation results in an increased abundance of α-ENaC protein in response to either exogenous aldosterone or dietary Na^+-Cl^- restriction[141]; the response to Na^+-Cl^- restriction is blunted by spironolactone, indicating involvement of the mineralocorticoid receptor. At the baseline, α-ENaC transcripts in the kidney are less abundant than those encoding β- and γ-ENaC. All three subunits are required for the efficient processing of heteromeric channels in the endoplasmic reticulum and trafficking to the plasma membrane, such that the induction of α-ENaC is thought to relieve a major bottleneck in the processing and trafficking of active ENaC complexes.[142]

Aldosterone also plays an indirect role in the regulated trafficking of ENaC subunits to the plasma membrane via the regulation of accessory proteins that interact with preexisting ENaC subunits. Aldosterone rapidly induces the expression of a serine-threonine kinase denoted SGK1.[143] The coexpression of SGK1 with ENaC subunits in *Xenopus* oocytes results in a dramatic activation of the channel due to increased expression at the plasma membrane.[141] Analogous in vivo redistribution of ENaC subunits occurs in the CNT and early CCD from a largely cytoplasmic location during dietary Na^+-Cl^- excess to a purely apical distribution after aldosterone or Na^+-Cl^- restriction.[71,141] Furthermore, there is a temporal correlation between the appearance of induced SGK1 protein in the CNT and the redistribution of ENaC protein to the plasma membrane.[141] The amiloride-sensitive, lumen-negative potential difference generated by ENaC is reduced in SGK1 knockout mice,[144] resulting in a decreased driving force for distal K^+ secretion and marked susceptibility to hyperkalemia.

SGK1 modulates membrane expression of ENaC by interfering with regulated endocytosis of its channel subunits. Specifically, the kinase interferes with interactions between ENaC subunits and the ubiquitin-ligase Nedd4-2.[142] PPxY domains in the C-termini of all three ENaC subunits bind to WW domains of Nedd4-2. Coexpression of Nedd4-2 with a wild-type ENaC channel results in a marked inhibition of channel activity due to retrieval from the cell membrane; Nedd4-2 is thought to ubiquitinate ENaC subunits, resulting in the removal of channel subunits from the cell membrane and degradation in lysosomes and the proteosome.[142] A PPxY domain in SGK1 also binds to Nedd4-2, which is a phosphorylation substrate for the kinase; the phosphorylation of Nedd4-2 by SGK1 abrogates its inhibitory effect on ENaC subunits.[145,146] Aldosterone also stimulates Nedd4-2 phosphorylation in vivo[147] and Nedd4-2 phosphorylation, in turn, results in the ubiquitin-mediated degradation of SGK1.[148]

Finally, aldosterone indirectly activates ENaC channels through the induction of channel activating proteases, which increase open channel probability by the cleavage of the extracellular domain of α- and γ-ENaC. Proteases that have been implicated in the processing of ENaC include furin, elastase, plasmin, kallikrein, and three novel, membrane-associated proteases called channel activating proteases 1, 2, and 3(CAP1, 2, and 3).[149–151] Proteolytic cleavage of ENaC appears to activate the channel by removing the self-inhibitory effect of external Na^+[150]; in furin-mediated proteolysis of αENaC, this appears to involve the removal of an inhibitory domain from within the extracellular loop.[152] The structures of the extracellular domains of ENaC and the related channels resemble an outstretched hand holding a ball, with defined subdomains termed the wrist, finger, thumb, palm, β-ball, and knuckle; functionally relevant proteolytic events target the finger domains of ENaC subunits.[151] Unprocessed channels at the plasma membrane are thought to function as a reserve pool, capable of rapid activation by membrane-associated luminal proteases.[150]

Adaptation to High Potassium Intake

Much of the renal adaptation to high K^+ intake is aldosterone independent. For example, a high K^+ diet increases apical Na^+ reabsorption and K^+ secretion in the CCD in adrenalectomized animals that lack aldosterone.[153] At the tubular level, when basolateral K^+ is increased, there is a significant activation of the Na^+/K^+–ATPase, accompanied by a secondary activation of apical Na^+ and K^+ channels.[154] Increased dietary K^+ also markedly increases the density of SK channels in the CCD (Fig. 6.5), along with a modest increase in ENaC density.[136] This is associated with changes in the subcellular distribution of the ROMK protein, with an increase in apical expression.[155] Notably, this increase in ENaC and SK density in the CCD occurs within hours of assuming a high K^+ diet, with a minimal associated increase in circulating aldosterone.[156] In contrast, a week of low Na^+-Cl^- intake, with almost a 1000-fold increase in aldosterone, has

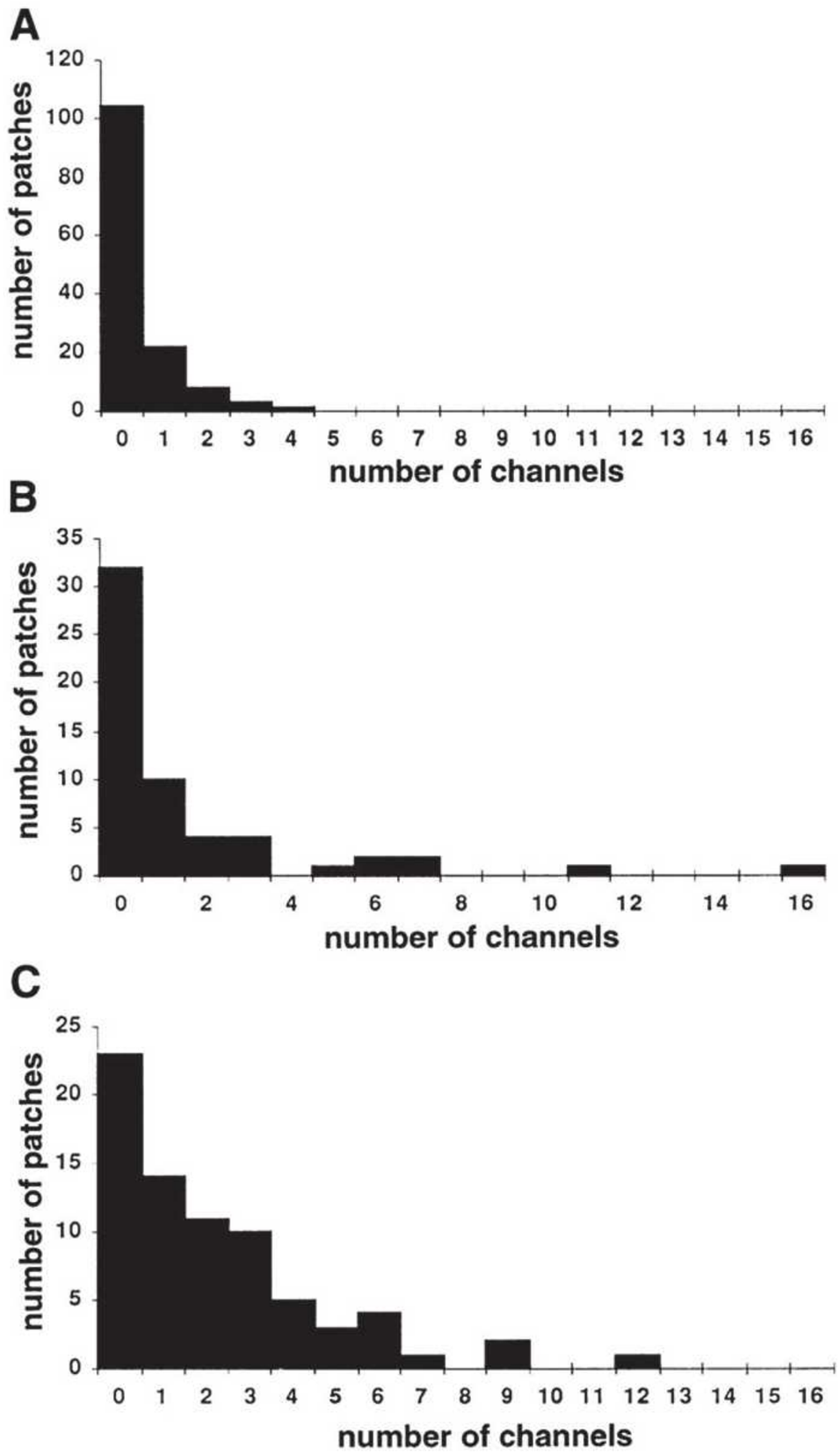

FIGURE 6.5 A high K^+ diet rapidly activates small conductance (SK) channels in the cortical collecting duct (CCD), which is mediated by the renal outer medullary K^+ channel (ROMK) (Kir 1.1) K^+ channel. Histograms of *N* (channels/patch) are shown for rats on a control diet (**A**), on a high K diet for 6 hours (**B**), and on a high K diet for 48 hours (**C**). Each determination of *N* represents a single cell-attached patch. The high K^+ diet results in a progressive recruitment of SK channels at the apical membrane. (From Palmer LG, Frindt G. Regulation of apical K channels in rat cortical collecting tubule during changes in dietary K intake. *Am J Physiol.* 1999;277:F805–812.)

no effect on SK channel density, nor for that matter does 2 days of aldosterone infusion, despite the development of hypokalemia (Table 6.1).[156] Of note, unlike the marked increase seen in the CCD,[136,156] the density of SK channels in the CNT is not increased by high dietary K^+.[65] This appears to be due to difficulties in estimating channel densities in small membrane patches, because the measurement of whole cell currents indicates an upregulation of ROMK activity in the CNT of animals maintained on a high K^+ diet.[157]

BK channels in the CNT and CCD play an important role in the flow-activated component of distal K^+ excretion[75]; these channels are also activated by dietary K^+ loading. Flow-stimulated K^+ secretion by the CCD of both mice[77] and rats[158] is enhanced on a high-K^+ diet, with an absence of flow-dependent K^+ secretion in rats on a low K^+ diet.[158] This is accompanied by commensurate changes in transcript levels for α- and β_{2-4}-subunits of the BK channel proteins in microdissected CCDs (β_1 subunits are restricted to the CNT[75]). Trafficking of BK subunits is also affected by dietary K^+, with largely intracellular distribution of α-subunits in K^+-restricted rats and prominent apical expression in K^+-loaded rats.[158] Aldosterone does not contribute to the regulation of BK channel activity or expression in response to a high K^+ diet.[159]

Regulatory information is notably more extensive for ROMK/SK channels than for BK channels. ROMK channels exhibit a high P_o; physiologic regulation of SK channels is primarily achieved by regulated changes in the number of active channels on the plasma membrane. The changes in trafficking and/or activity of the ROMK channel that are induced by dietary K^+ restriction appear to involve tyrosine phosphorylation of the ROMK protein (see the following); K^+ loading reverses this tyrosine phosphorylation. The forward trafficking of ROMK channels to the apical membrane of CNT and principal cells after K^+ loading is also phosphorylation dependent. PKA phosphorylates ROMK protein at one N-terminal serine and two C-terminal serines.[124] Phosphorylation of the N-terminal serine overrides the effect of a carboxy-terminal endoplasmic reticulum retention signal, thus inducing exit from the Golgi apparatus and expression of the channel protein at the cell membrane.[125] This explains in large part the induction of SK channel density by vasopressin[160] and cAMP.[76] Notably, this N-terminal serine is also a substrate for SGK1, the aldosterone-induced kinase that activates ENaC, which then activates ROMK by increasing membrane expression.[161] Thus, although aldosterone does not appear to have significant effects on SK density,[156] SGK1 is conceivably involved; alternatively, other antagonistic effects of SGK1 on ROMK[162] trafficking abrogate the effect mediated by N-terminal phosphorylation.

A recent series of reports have linked changes in the expression of WNK1 kinase subunits in the response to high K^+ diet. WNK1 and WNK4 were initially identified as causative genes for familial hypertension with hyperkalemia (FHHt), also known as Gordon syndrome or pseudohypoaldosteronism type II.[163] The regulation of NCC, the thiazide-sensitive Na^+-Cl^- cotransporter in the DCT, is a major role of the WNK kinases.[164–166] NCC activity is a major determinant of Na^+ delivery to the downstream CNT, with potent effects on the ability to secrete K^+ (see also Integrated Regulation of Distal Sodium and Potassium Transport). However, the WNK kinases also directly regulate trafficking and activity of both ROMK and BK channels.

ROMK expression at the membrane of Xenopus oocytes is dramatically reduced by the coexpression of WNK4; FHHt-associated mutations dramatically increase this effect, suggesting a direct inhibition of SK channels in FHHt.[167]

6.1 The Effect of a High K^+ Diet, Aldosterone, and/or Na^+-Cl^- Restriction on Small Conductance Channel Density in the Rat Cortical Collecting Duct

Condition	K Channel Density/μm^2	Plasma Aldo (ng/dL)	Plasma K (mM)
Control	0.41	15	3.68
High K diet, 6 h	1.51	36	NM
High K diet, 48 h	2.13	98	4.37
Low Na diet, 7 d	0.48	1260	NM
Aldo infusion, 48 h	0.44	550	2.44
Aldo + high K diet	0.32	521	3.80

K, potassium; h, hour; Na, sodium; d, day; Aldo, aldosterone; NM, not measured.
Modified from Palmer LG, Frindt G. Regulation of apical K channels in rat cortical collecting tubule during changes in dietary K intake. Am J Physiol. 1999;277:F805–F812.

The coexpression of WNK4 also inhibits BK channels in a heterologous expression system, apparently by inducing lysosomal degradation.[168] In the case of WNK1, the analysis is complicated dramatically by the transcriptional complexity of its gene, which has at least three separate promoters and a number of alternative isoforms. In particular, the predominant intrarenal WNK1 isoform is generated by a distal nephron transcriptional site that bypasses the N-terminal exons that encode the kinase domain, thus yielding a kinase-deficient short form of the protein ("WNK1-S").[169] Full-length WNK1 (WNK1-L) inhibits ROMK activity by inducing endocytosis of the channel protein[170–172]; kinase activity and/or the N-terminal kinase domain of WNK1 appear to be required for this effect,[171,172] although Cope et al.[170] have reported that a kinase-dead mutant of WNK1 is unimpaired. WNK1 and WNK4 induce endocytosis of ROMK via an interaction with intersectin, a multimodular endocytic scaffold protein.[173] The additional binding of ROMK to the clathrin adaptor protein autosomal recessive hypercholesterolemia (ARH) is required for basal and WNK1-stimulated endocytosis of the channel protein.[174] The ubiquitination of the ROMK protein is also involved in clathrin-dependent endocytosis, requiring an interaction between the channel and the U3 ubiquitin ligase POSH (plenty of SH domains).[175]

The shorter WNK1-S isoform, which lacks the kinase domain, appears to inhibit the effect of WNK1-L.[171,172] The ratio of WNK1-S to WNK1-L transcripts is reduced by K^+ restriction (greater endocytosis of ROMK)[172,176] and is increased by K^+ loading (reduced endocytosis of ROMK),[171,176] thus suggesting that this ratio between WNK1-S and WNK1-L functions as a "switch" to regulate distal K^+ secretion (Fig. 6.6). The inhibitory effect of WNK1-S tracks to the first 253 amino acids of the protein, encompassing the initial 30

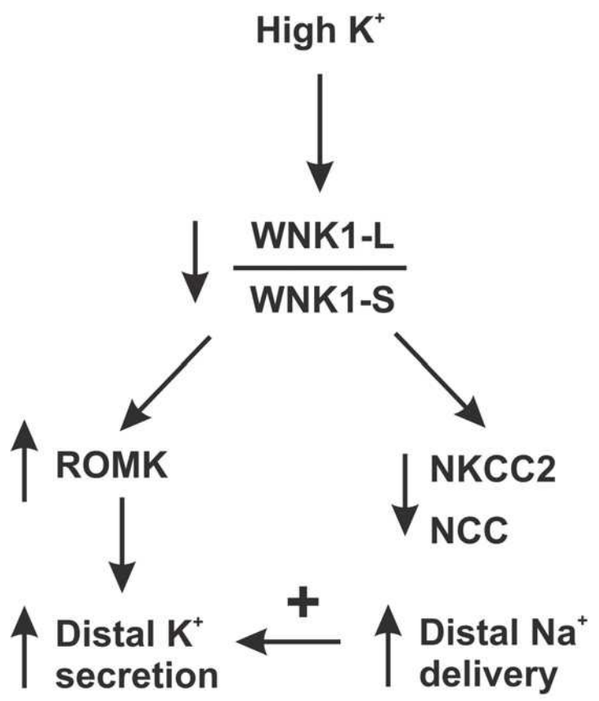

FIGURE 6.6 The balance between the major short (WNK1-S) and long (WNK1-L) isoforms of the WNK1 kinases affects K^+ secretion. The WNK1-S isoform lacks a kinase domain and inhibits the effects of the long isoform. Potassium loading causes a reduction in the ratio of WNK1-L to WNK1-S, resulting in an increased expression of the renal outer medullary K^+ channel (ROMK) at the plasma membrane of connecting tubule (CNT) cells and principal cells, with a concomitant reduction in the apical expression of the thiazide-sensitive Na^+-Cl^- cotransporter (NCC) in the distal convoluted tubule (DCT) and the furosemide-sensitive Na^+-K^+-$2Cl^-$ cotransporter NKCC2 in the thick ascending limb (TAL). The net result is increased Na^+ delivery to the CNT and principal cells with enhanced apical secretory K^+ channel activity, resulting in an enhanced K^+ secretion.

amino acids unique to this isoform and an adjacent autoinhibitory domain.[177] Transgenic mice that overexpress this inhibitory domain of WNK1-S have lower serum K^+ concentrations, higher fractional excretion of K^+, and increased expression of the ROMK protein at the apical membrane of CNT and CCD cells, all of which are consistent with an important inhibitory effect of WNK1-S.[177]

Potassium Deprivation

A reduction in dietary K^+ leads, within 24 hours, to a dramatic drop in urinary K^+ excretion.[176,178] This drop in excretion is due to both an induction of reabsorption by intercalated cells in the OMCD[107,108] and to a reduction in SK channel activity in principal cells.[179] The mechanisms involved in K^+ reabsorption by intercalated cells were previously discussed. Notably, H^+/K^+–ATPase activity in the collecting duct does not appear to be regulated by aldosterone[180]; stimulatory effects of mineralocorticoid on H^+/K^+–ATPase activity are abrogated by K^+ loading, indicating a primary role for hypokalemia.[181]

Dietary K^+ intake modulates trafficking of the ROMK channel protein to the plasma membrane of principal cells, with a marked increase in the relative proportion of intracellular channel protein in K^+-depleted animals[155] and clearly defined expression at the plasma membrane of CCD cells from animals on a high K^+ diet.[155] The membrane insertion and activity of ROMK is modulated by tyrosine phosphorylation of the channel protein, such that the phosphorylation of tyrosine residue 337 stimulates endocytosis and dephosphorylation induces exocytosis.[182,183] This tyrosine phosphorylation appears to play a key role in the regulation of ROMK by dietary K^+.[184] Whereas the levels of protein tyrosine phosphatase-1D do not vary with K^+ intake, intrarenal activity of the cytoplasmic tyrosine kinases c-src and c-yes are inversely related to dietary K^+ intake, with a decrease under high K^+ conditions and a marked increase after several days of K^+ restriction.[179,185] Immunolocalization indicates coexpression of c-src with ROMK in the TAL and the principal cells of the CCD.[155] The inhibition of protein tyrosine phosphatase activity, leading to a dominance of tyrosine phosphorylation, dramatically increases the proportion of intracellular ROMK in the CCD of animals on a high K^+ diet.[155]

The neurohumoral factors that induce the K^+-dependent trafficking and expression of apical ROMK[155] and BK channels[158] have only recently been identified. Several studies have implicated the intrarenal generation of superoxide anions in the activation of cytoplasmic tyrosine kinases and in the downstream phosphorylation of the ROMK channel protein by K^+ depletion.[186,187] Candidates for the upstream kaliuretic factor include AT-II and growth factors such as insulin-like growth factor-1 (IGF-1).[162] AT-II inhibits ROMK activity in K^+-restricted rats, but not in rats on a normal K^+ diet.[188] This inhibition involves the downstream activation of superoxide production and c-src activity, such that the well-known induction of AT-II by a low K^+ diet appears to play a major role in reducing the distal tubular K^+ secretion.[189] IGF-1 is produced in the kidney and upregulated by dietary K^+ restriction[190]. The downstream activation of PI3-kinase by insulin and IGF-1 results in the Akt1- or SGK1-dependent phosphorylation of WNK1, leading to endocytosis of ROMK.[162]

Reports of transient postprandial kaliuresis in sheep, independent of changes in plasma K^+ or aldosterone, suggest that an enteric or hepatoportal K^+ sensor controls kaliuresis via a sympathetic reflex[191]; tissue kallikrein has recently emerged as a candidate mediator for this postprandial kaliuresis (see the following). Regardless of the signaling involved, changes in dietary K^+ absorption have a direct "anticipatory" effect on K^+ homeostasis in the absence of changes in plasma K^+. Such a feed-forward control has the theoretical advantage of greater stability, because it operates prior to changes in plasma K^+.[192] Notably, changes in ROMK phosphorylation status and insulin-sensitive muscle uptake can be seen in K^+-deficient animals in the absence of a change in plasma K^+,[13] suggesting that the upstream activation of the major mechanisms that serve to reduce K^+ excretion (reduced K^+ secretion in the CNT/CCD, decreased peripheral uptake, and increased K^+ reabsorption in the OMCD) does not require changes in the plasma K^+. Consistent with this hypothesis, moderate K^+ restriction—without an associated drop in plasma K^+—is sufficient to induce AT-II-dependent superoxide generation and c-src activation, leading to the inhibition of ROMK channel activity.[189]

Vasopressin

Vasopressin has a stimulatory effect on K^+ secretion by the distal nephron.[32] This serves to preserve K^+ secretion during dehydration and extracellular volume depletion, when circulating levels of vasopressin are high and tubular delivery of Na^+ and fluid is reduced. The stimulation of basolateral V2 receptors results in the activation of ENaC, which increases the driving force for K^+ secretion by CNT cells and principal cells.[193] In addition, vasopressin activates SK channels directly in the CCD,[160] as does cAMP.[76] The ROMK protein is directly phosphorylated by PKA on three serine residues (S25, S200, and S294 in the ROMK2 isoform), with phosphorylation of all three sites required for full activity (see also the previous discussion). Finally, the stimulation of luminal V1 receptors also increases K^+ secretion in the CCD, apparently via the activation of BK channels.[194]

Tissue Kallikrein

The serine protease tissue kallikrein (TK) is involved in the generation of kinins, which ultimately stimulates the formation of bradykinin.[195] Within the kidney, TK is synthesized within CNT cells and is released into the tubular lumen and the peritubular interstitium. Although TK-induced bradykinin has a number of effects on distal tubular physiology,[195] more recent data have revealed a provocative role in postprandial kaliuresis. Thus, oral K^+-Cl^- loading leads to a spike in urinary K^+ and TK excretion in rats, mice, and

humans.[195] The increase in urinary TK after K^+ loading is not accompanied by changes in urinary aldosterone and can be detected in aldosterone synthase knockout mice.[149] Mice deficient in TK demonstrate postprandial hyperkalemia, indicating a role for the protease in postprandial kaliuresis. This transient hyperkalemia is accompanied by a marked increase in K^+ reabsorption by perfused CCDs due to an upregulation of H^+/K^+–ATPase activity and an increase in HKα-2 transcript. The addition of luminal but not basolateral TK inhibits the activated CCD H^+/K^+–ATPase activity in TK knockout mice, which is consistent with direct proteolytic activation. There is also a marked increase in Na^+ reabsorption by perfused CCDs from TK knockout mice, without the development of a lumen-negative PD. This is consistent with an increased activity of the electroneutral Na^+-Cl^- cotransport mediated by the Na^+-driven SLC4A8 Cl^--HCO_3^- exchanger and the SLC26A4 Cl^--HCO_3^- exchanger (see also the following text).[196] This electroneutral transport pathway had previously been shown to be inhibited by bradykinin[197]; hence, the activation by TK deletion presumably reflected a loss of tonic inhibition by TK-generated bradykinin. Prior data had indicated that TK mediates proteolytic cleavage of the γ subunit of ENaC, with reduced ENaC activity in TK-deficient mice[198]; net Na^+ balance is thus neutral in these mice.

In summary, TK secretion from CNT cells is induced by oral K^+-Cl^- loading, causing proteolytic activation of ENaC[198] and thus an increase in ENaC-driven K^+ secretion and the bradykinin-dependent inhibition of electroneutral Na^+-Cl^- cotransport in the CCD.[196,197] Thus, a further augmentation of electrogenic Na^+ transport (favoring K^+ secretion) and a direct luminal inhibition of H^+/K^+–ATPase activity causes a decrease or tonic inhibition of K^+ reabsorption. TK may very well be the postprandial factor[191] that functions in the feed-forward control of plasma K^+.[192]

The Integrated Regulation of Distal Sodium and Potassium Transport

In CNT and principal cells, the lumen-negative potential difference generated by Na^+ entry via ENaC induces the exit of K^+ via apical K^+-selective channels. This arrangement explains much of the known physiology and pathophysiology of renal K^+ secretion, yet has several key consequences that bear emphasis. First, enhanced Na^+-Cl^- reabsorption upstream of the CNT and CCD will reduce the delivery of luminal Na^+ to the CNT and CCD,[73] decrease the lumen-negative potential difference, and thus decrease K^+ secretion; K^+ secretion by the CCD essentially stops when luminal Na^+ drops below 8 mmol per liter.[74] In this respect, the increasingly refined phenotypic understanding of FHHt, caused by kinase-induced gain-of-function of the DCT, has served to underline that a variation in NCC-dependent Na^+-Cl^- absorption, just upstream of the CNT, has major effects on the ability to excrete dietary K^+.[166] Second, aldosterone is a kaliuretic hormone, induced by hyperkalemia. However, in certain circumstances associated with a marked induction of aldosterone, such as dietary sodium restriction, sodium balance is maintained without affecting K^+ homeostasis. This aldosterone paradox—the independent regulation of Na^+-Cl^- and K^+ handling by the aldosterone-sensitive distal nephron—is only recently beginning to yield to investigative efforts. The major factors in the integrated control of Na^+-Cl^- and K^+ transport appear to include electroneutral, NCC-independent, and thiazide-sensitive Na^+-Cl^- transport within the CCD[196,197,199]; K^+-dependent changes in NCC activity within the DCT; ENaC-independent K^+ excretion within the distal nephron[72]; and the differential regulation of various transport and signaling pathways by aldosterone, AT-II, TK, and dietary K^+.[195,200,201]

Thiazide-sensitive electroneutral Na^+-Cl^- transport within the CCD is mediated by parallel activity of the Na^+-driven SLC4A8 Cl^--HCO_3^- exchanger and the SLC26A4 Cl^--HCO_3^- exchanger,[196] expressed in intercalated cells. The molecular identity of this transport mechanism has only recently emerged[196]; hence, regulatory influences are not fully characterized. However, electroneutral Na^+-Cl^- transport within the CCD is evidently induced by both volume depletion and mineralocorticoid treatment.[196,197,199] This mechanism mediates ~50% of Na^+ reabsorption in the CCD under these conditions, all without affecting the lumen potential difference and thus without influencing K^+ excretion. A high K^+ diet also increases the fraction of ENaC-independent, amiloride-resistant K^+ excretion to ~50%; again, this electroneutral, aldosterone-independent pathway for K^+ excretion uncouples distal tubular Na^+ and K^+ excretion.[72]

In a landmark study, Kahle et al.[167] established in 2003 that the WNK4 kinase, encoded by a disease gene for FHHt, inhibits ROMK activity in Xenopus oocytes. This identified WNK-dependent signaling as a major pathway for integrating Na^+-Cl^- and K^+ transport within the distal nephron. Details of the relevant effects of WNK kinases on NCC and ROMK are discussed earlier and elsewhere.[202–204] However, key findings include the differential influence of K^+ intake on circulating AT-II, ROMK activity (i.e., K^+ secretory capacity), the ratio of WNK1 isoforms, and the activity of NCC in the DCT. AT-II activates NCC via WNK4 and the downstream SPAK kinase,[202–204] thus reducing delivery of Na^+ to the CNT and limiting K^+ secretion. AT-II also inhibits ROMK activity via several mechanisms, including the downstream activation of c-src tyrosine kinases.[187–189] Whereas K^+ restriction induces renin and circulating AT-II, increases in dietary K^+ suppress AT-II levels.[189,205] A decrease in circulating and local AT-II explains why NCC phosphorylation and activity is downregulated by a high K^+ diet (Fig. 6.7).[56,206] Teleologically, this serves to increase the delivery of Na^+ to the CNT, thus increasing K^+ secretion. Finally, within CNT cells and principal cells, increases in aldosterone induce the SGK1 kinase, which phosphorylates WNK4 and attenuates the effect of WNK4 on ROMK,[207] while activating ENaC via Nedd4-2-dependent effects. However, when dietary K^+ intake is reduced, c-src tyrosine kinase activity increases under

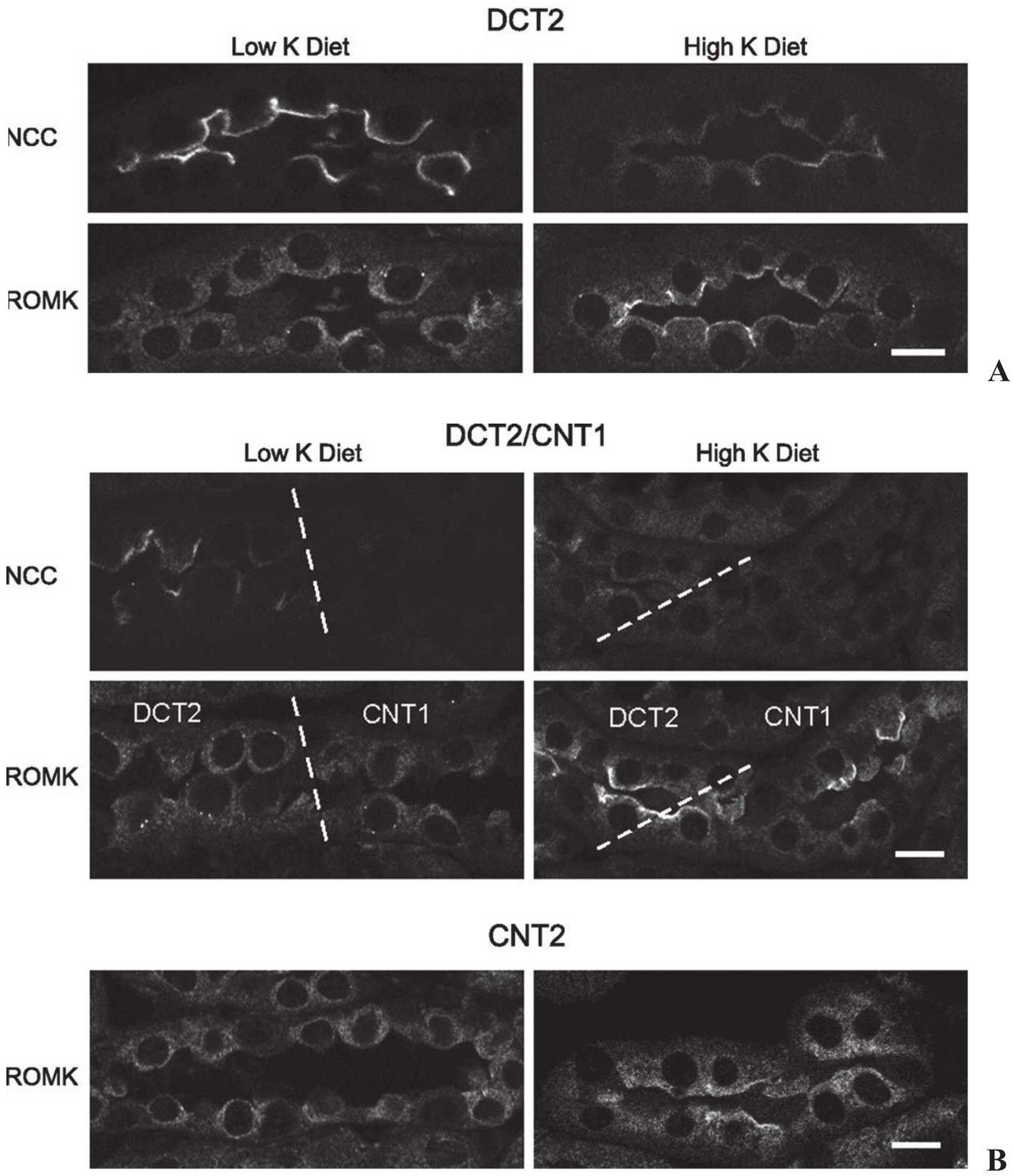

FIGURE 6.7 The differential effects of a high and low K^+ diet on the membrane expression of Na^+-Cl^- cotransporter (NCC) and renal outer medullary K^+ channel (ROMK) in late distal convoluted tubule (DCT) and the connecting tubule (CNT). **A:** Whereas NCC expression at the plasma membrane of DCT2 cells is enhanced in rats treated with a low K^+ diet and is reduced by a high K^+ diet, the opposite occurs for ROMK protein. **B:** This dichotomy persists at the junction between DCT2 and the early CNT (CNT1: the juncture is denoted by a *dashed white line*). However, within CNT2 segments, which lack NCC, apical labeling of ROMK under high K^+ conditions is less striking. (From Wade JB, et al. Differential regulation of ROMK (Kir1.1) in distal nephron segments by dietary potassium. *Am J Physiol.* 2011;300(6):F1385–1393.)

the influence of increased AT-II, causing direction inhibition of ROMK activity via tyrosine phosphorylation of the channel.[182–184] The increase in c-src tyrosine kinase activity also abrogates the effect of SGK1 on WNK4[201]; this latter mechanism can also be induced by AT-II.[208]

The ratio of WNK1 isoforms is also a critical determinant in the balance between distal Na^+ and K^+ transport. The shorter WNK1-S isoform, which lacks the kinase domain, appears to inhibit the effect of WNK1-L.[171,172] The ratio of WNK1-S to WNK1-L transcripts is reduced by K^+ restriction (greater endocytosis of ROMK)[172,176] and is increased by K^+ loading (reduced endocytosis of ROMK).[171,176] This suggests that the ratio between WNK1-S and WNK1-L functions as a switch to regulate distal K^+ secretion (see also Fig. 6.6). Transgenic mice that overexpress this inhibitory domain of WNK1-S have lower serum K^+ concentrations, a higher fractional excretion of K^+, and an increased expression of ROMK protein at the apical membrane of CNT and CCD cells, all of which are consistent with an important inhibitory effect of WNK1-S.[177] In contrast, WNK1-L activates NCC by enhancing expression at the cell membrane; this activation is blocked by WNK1-S. Again, transgenic WNK1-S mice have the expected phenotype, with renal salt wasting and hypotension due to marked suppression of membrane-associated total and phosphorylated NCC in the DCT and NKCC2 in the TAL.[209] In contrast, selective knockout of the WNK1-S exons leads to a major gain in function for NCC and NKCC2.[209] Therefore, K^+ loading will lead to an increase in the ratio of WNK1-S to WNK1-L, reduced endocytosis of ROMK, and enhanced endocytosis of NCC; this will have the effect of reducing Na^+ absorption in the DCT and thus increasing Na^+ delivery and tubular flow rate in the CNT, where ROMK +/– BK channel activity is maximized (see also Fig. 6.6). The converse cascade occurs in K^+ restriction.

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