

Chapter 2

Fundamentals of Epithelial Na⁺ Absorption

Alexander Staruschenko, Daria V. Ilatovskaya, and Kenneth R. Hallows

Abstract The maintenance of electrolyte balance is essential for the control of many functions in the human body. Na⁺, K⁺, and Cl⁻ are key electrolytes that contribute to a variety of processes ranging from the maintenance of cellular membrane potential to the regulation of cell volume and extracellular fluid volume. Fundamentals of epithelial Cl⁻ and K⁺ transport are discussed in the preceding and following chapters and will be only briefly touched upon here. Na⁺ absorption occurs across the epithelial barriers of many organs, including the lung, gastrointestinal tract, exocrine glands, and kidney. Na⁺ is the primary determinant of blood volume, and a number of physiological mechanisms that control blood pressure mediate their effects by adjusting Na⁺ balance in the kidney. This chapter describes classical fundamentals of epithelial Na⁺ absorption and highlights some recent mechanisms involved in physiological regulation of Na⁺ transport in specific epithelia with a particular emphasis on the kidney.

Keywords Kidney • Hypertension • Na⁺/K⁺ pump • NHE • SGLT • NKCC • NCC • ENaC • NBC • NPT

2.1 Introduction

In this book, specific chapters deal with all aspects of transport of the individual solutes and electrolytes. In the present chapter, we focus on general principles of epithelial Na⁺ absorption and mechanisms controlling this process. As recently summarized in an excellent review by Kotchen and colleagues, current levels of salt

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consumption in modern society generally exceed total body salt needs and are associated with adverse clinical outcomes. High salt intake is associated with hypertension and increased rates of cardiovascular disease. Experimental studies continue to provide information about mechanisms for these adverse effects of salt (Kotchen et al. 2013). Thus, despite the critical role of NaCl in the maintenance of multiple mechanisms in the human body, many individuals, especially those on Western diets, need to limit salt consumption. When we eat too much salt, several organs, especially the kidneys, act to help us excrete the excess salt load. Sodium absorption in the kidney is precisely regulated and controlled by numerous mechanisms, many of which are reviewed in this chapter. Multiple studies have focused on transport processes in the kidney. As a consequence, much of our current knowledge of the principles of epithelial transport has been derived from studies on renal epithelia. Thus, we provide here the fundamentals of epithelial Na⁺ absorption, focusing primarily on the mechanisms involved in the transport of this important ion in the kidney. In addition, modulation of transepithelial Na⁺ reabsorption in the lung plays a key role in determining airway surface liquid volume and airway clearance, which is dysregulated in certain diseases like cystic fibrosis. The following section of this chapter will briefly describe sodium transport in the lung and other organs.

Although our understanding of the specific details and mechanisms of function of epithelial Na⁺ absorption has greatly expanded recently, the main concepts remain remarkably similar to those described 50–60 years ago. Several excellent review articles and book chapters have described these mechanisms, and we briefly summarize them here. In addition, we recommend to the reader additional renal physiology textbooks, including those edited by Schrier, Brenner, Boron, and Guyton/Hall.

Na⁺ is the principal extracellular cation and is present within cells at a much lower concentration than it is in the extracellular space. Typically, Na⁺ concentrations are 10–20 mM and 135–145 mM in the intracellular and extracellular compartments in humans, respectively. The glomeruli filter approximately 25,000 mEq of Na⁺ per day. As sodium is freely filtered by glomeruli, the kidneys must reabsorb the vast majority of Na⁺ as the filtrate flows along the nephron. Na⁺ absorption in the kidney is co-regulated with the transport of other ions (including chloride, phosphate, bicarbonate, potassium, protons, calcium, and magnesium), neutral solutes (e.g., glucose and amino acids), and water via directly or functionally coupled transport processes at both the apical and basolateral membranes. This is accomplished by the integrated function of consecutive sodium transporters and channels along the nephron.

2.2 General Concepts of Sodium Absorption in Epithelia

2.2.1 Basic Principles of Sodium Transport

Epithelial sodium (Na⁺) absorption represents in general reabsorption of sodium ions from the tubules back into the blood. In this case, it is called reabsorption rather than absorption because sodium returns to the circulation after it had been secreted the renal tubules. The classical concept of transepithelial NaCl absorption, which was formulated more than 50 years ago by Koefoed-Johnsen and Ussing for the frog skin (Koefoed-Johnsen and Ussing 1958) is still valid in general for sodium reabsorption in the kidney. Palmer and Andersen briefly discussed the historical background and wide-ranging impact and implications of this seminal work (Palmer and Andersen 2008).

In the kidney, Na⁺, along with other ions such as chloride (Cl⁻) and bicarbonate (HCO₃⁻), is highly reabsorbed. However, the rate of reabsorption depends on body needs and is precisely controlled to maintain homeostatic balance. To be reabsorbed, Na⁺ must be transported across the tubular epithelial membranes in processes that will be discussed in this chapter. Reabsorption of Na⁺ across the tubular epithelium into the interstitium includes active transport, passive diffusion, or both. The rate of total transport across a cellular membrane (dM/dt) is the sum of passive (PT) and active (carrier-mediated; CM) transport and can be described by using the Michaelis–Menten Eq. (2.1) (Sugano et al. 2010):

$$\frac{dM}{dt} = A \times (P_{PT} + P_{CM}) \times C = A \times P_{PT} \times C + \frac{V_{\max} \times C}{K_m + C} \quad (2.1)$$

where A is the surface area of a membrane (length²), P is the permeability (length/time), C is the concentration of a permeant species (amount/length³), K_m is the Michaelis constant (amount/length³), and V_{\max} is the maximum carrier-mediated transport (amount/time) (Sugano et al. 2010).

As shown in Fig. 2.1, there are two basic mechanisms: (1) the transcellular route whereby sodium is transported through the cell across both the apical and basolateral plasma membranes and (2) the paracellular route, where sodium ions traverse the spaces between cell–cell junctions. After Na⁺ is reabsorbed across the tubular epithelial cells into the interstitial fluid, sodium is then transported through peritubular capillary walls into the blood by ultrafiltration. This process is mediated by Starling forces (Starling 1896; Levick and Michel 2010), a combination of hydrostatic pressure and colloid osmotic forces, which will not be covered further in this chapter.

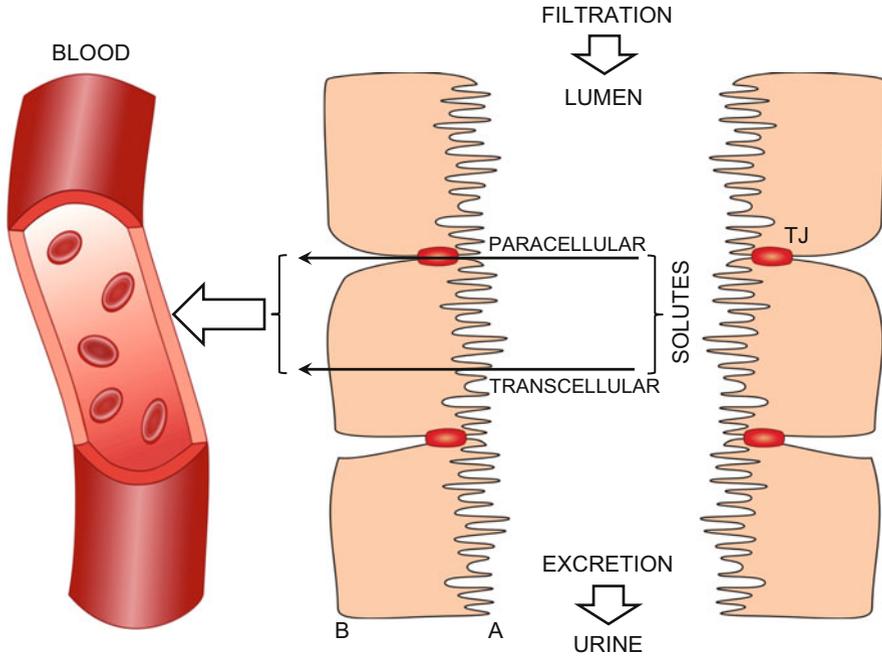


Fig. 2.1 General concept of active and passive Na⁺ transport through the cell. Reabsorption of filtered Na⁺ from the tubular lumen across the epithelial cells back into the blood. Na⁺ is transported either by active transport through the cells (transcellular pathway) or between the cells (paracellular pathway) by diffusion. Tight junctions (TJ) are shown

2.2.2 Cytosolic Diffusion

As described in Einstein's theory of Brownian motion, molecular diffusion (or diffusion) consists of the random movements of a molecule with respect to adjacent molecules and occurs as the consequence of thermal energy. Since the diffusional movement of an individual molecule is random, a concentration gradient is required for any net transfer of molecules to occur across a membrane. Thus, the concentration gradient represents a major driving force for net transport. In addition, the movements of charged solutes (ions) are governed by electrical gradients created by potential differences between compartments, and thus net ion fluxes are dictated by their electrochemical gradients across membranes.

2.2.3 Maintenance of Membrane Potential

In addition to its critical role in the maintenance of electrolyte homeostasis, Na⁺ concentrations play an important role in the maintenance of cellular membrane

potential, as originally formulated by Goldman, Hodgkin, and Katz (Goldman 1943; Hodgkin and Katz 1949). Studies of various segments of the nephron have revealed heterogeneity of the electrical properties along the segments. The electrophysiological properties, such as transepithelial voltage and resistance (V_t and R_T , respectively), vary widely. Na⁺, along with K⁺ and Cl⁻, contributes to the maintenance of concentration and charge differences across cell membranes. The intracellular and extracellular concentration differences between K⁺ and Na⁺ create an electrochemical gradient across the plasma membrane known as the cellular membrane potential. In kidney epithelial cells, the cellular membrane potential is maintained mostly by ionic gradients generated by the Na⁺/K⁺-ATPase and governed by the relative permeabilities of the major permeant ions, K⁺, Cl⁻, and Na⁺, through the ensemble of channels and transporters present in each cell type. As tight control of the cellular membrane potential is recognized as being critical in other organs such as brain, heart, and muscle, the maintenance of membrane potential in renal epithelial cells is also important for the coordinated transport of solutes and overall kidney function.

For charged solutes, the driving force for transport is the sum of the chemical and electrical potential gradients. The Nernst Eq. (2.2) describes the equilibrium condition for a membrane permeable only to a single ionic species:

$$V_m = V_2 - V_1 = \frac{RT}{ZF} \ln \frac{C_o}{C_i} \quad (2.2)$$

where R is the gas constant, T is the absolute temperature, Z is the valence of the solute, F is the Faraday constant, and C and V are concentration and electrical potential terms, respectively. At equilibrium, then, the voltage (V_m) across an ideally selective membrane is defined by the concentrations of the permeant ion on the inside and outside of the membrane, C_i and C_o , respectively.

For systems containing more than one permeant ion, i.e., almost all real physiological systems, the equilibrium membrane potential (V_m) can be described by the Goldman–Hodgkin–Katz (GHK) Eq. (2.3; equation is shown for Na⁺, K⁺, and Cl⁻):

$$V_m = \frac{RT}{F} \ln \left[\frac{(P_{Na}C_{Na}^o + P_KC_K^o + P_{Cl}C_{Cl}^i)}{(P_{Na}C_{Na}^i + P_KC_K^i + P_{Cl}C_{Cl}^o)} \right]. \quad (2.3)$$

where P_x is the permeability of the respective solutes, such as Na⁺, K⁺, and Cl⁻. Thus, in a polarized epithelial system containing multiple charged solutes, one can estimate the transmembrane voltages at both membranes as a function of the relative transmembrane concentrations and permeabilities of each solute on both sides of the apical and basolateral membranes.

2.2.4 Mechanisms of Na^+ Transport Across the Plasma Membrane

Na^+ absorption in the kidney includes a series of transport mechanisms. Active transcellular transport is the main mechanism for Na^+ absorption along the nephron. However, passive paracellular transport of Na^+ at least partially mediates sodium reabsorption in the kidney. Thus, in the proximal convoluted tubules (PCT) and thick ascending limb (TAL) of Henle's loop, sodium reabsorption takes both transcellular and paracellular routes (Breiderhoff et al. 2012; Muto et al. 2012). Passive paracellular transport is the predominant route for passive ion flows in the PCT where the paracellular resistance is low. In the case of active transport, Na^+ ions have to cross both the apical and basolateral membranes (or vice versa) in series. Due to hydrophobic properties of the membrane lipids, electrolytes such as Na^+ cannot cross the membrane freely and have to interact with specific transport proteins, which will be discussed in corresponding sections. Many of these transport proteins are being identified on a molecular basis, and our knowledge about these transport mechanisms has significantly improved in recent years.

2.2.4.1 Active Transcellular Transport

The coupling of solute transport to an energy source can take two forms: primary active transport, where solute transport is coupled directly to an energy-yielding reaction, and secondary active transport, where solute movement against its electrochemical gradient is energized by the movement of another solute down its own gradient.

The most common example of primary active transport is the transport of Na^+ and K^+ by the Na^+/K^+ -ATPase (also often referred to as the sodium pump or Na^+/K^+ pump), which is an electrogenic transmembrane ATPase mediating the exchange of 3 Na^+ ions outward for 2 K^+ ions inward at the expense of ATP hydrolysis with each cycle. Therefore, the pump is electrogenic. The Na^+/K^+ pump is localized at the basolateral membrane of many epithelial cells in different segments of the kidney, but it is especially abundant in the TAL of Henle's loop, cells of the distal convoluted tubule (DCT), connecting tubule (CNT) cells, and principal cells of the collecting duct (CD). In general, segments with high rates of active Na^+ transport have high Na^+/K^+ pump activity (Feraille and Doucet 2001). Na^+/K^+ pump functions as a heterodimeric protein complex comprised of catalytic α - and auxiliary β -subunits. The Na^+/K^+ pump is responsible for maintaining the intracellular Na^+ activity at a low level, which provides the energy for the Na^+ -coupled transport of many other solutes into the cell. More specific details on the structure and function of the Na^+/K^+ pump are provided in Chap. 11.

In contrast to primary active transport, in secondary active transport, also known as coupled transport, stored energy is used to transport molecules across a membrane instead of direct coupling to ATP hydrolysis. Na^+ , because of its steep inward electrochemical gradient maintained by the Na^+/K^+ pump, often participates in the

transport of other solutes. Depending on the direction of the movement of coupled solutes (same or opposite), secondary active transporters are classified either as cotransporters (or symporters) or exchangers (or antiporters). Robert K. Crane first proposed flux coupling in 1960 suggesting a model for the sodium–glucose cotransporter (SGLT; described in Sect. 2.3.2.1) (Crane 1960). Multiple cotransporters and exchangers involved in Na^+ absorption are expressed in epithelial cells. Specific transporters mediating Na^+ influx in exchange for efflux of other solutes are discussed below in sections describing Na^+ transport in defined segments.

There are multiple electrogenic Na^+ -driven cotransporters expressed in epithelial cells in the kidney, such as Na^+ -coupled HCO_3^- transporters (Parker and Boron 2013). In addition to electrogenic transporters, Na^+ entry is also mediated by electroneutral (i.e., when no net charge is transported across the membrane) Na^+ / H^+ exchangers (e.g., NHE3) and Na^+ -coupled cotransporters (e.g., NCC and NKCC). Shown on Fig. 2.2 are examples of both primary and secondary active

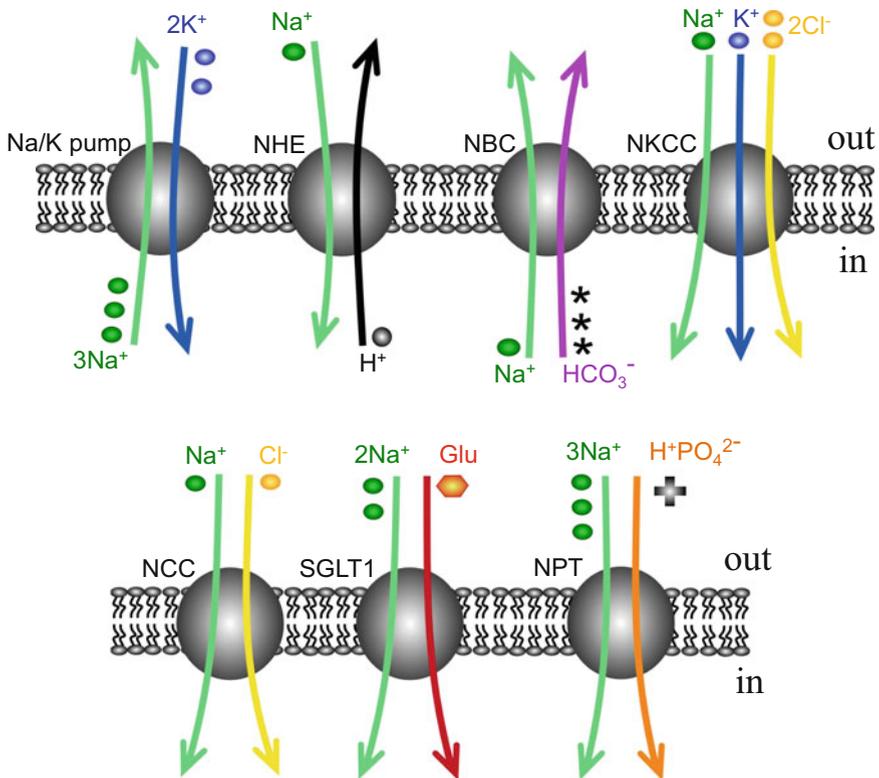


Fig. 2.2 Ion movement in the main Na^+ transporters involved in epithelial sodium absorption. Shown are primary transport pathways for Na^+/K^+ -ATPase, Na^+/H^+ exchanger (NHE), $\text{Na}-\text{HCO}_3$ cotransporter (NBC), $\text{Na}^+/\text{K}^+/\text{Cl}^-$ cotransporter (NKCC), Na^+/Cl^- cotransporter (NCC), sodium–glucose cotransporter (SGLT), and Na^+ -phosphate cotransporter (NPT). *Arrows* demonstrate direction of ion movement inside or outside of the cell

transporters mediating epithelial Na^+ absorption. As seen from Fig. 2.2, ion/substrate coupling stoichiometry of the transporters varies among transporters. For instance, the coupling stoichiometry of the SGLT1 cotransporter is 2 Na^+ ions to 1 glucose molecule per transport cycle (interestingly, SGLT1 stoichiometry is different from SGLT2, which is 1 Na^+ :1 glucose), but for NKCC 1 Na^+ ion is transported in the same direction as 1 K^+ and 2 Cl^- ions. Importantly, due to their various nature and function, they have different topologies in plasma membranes. Figure 2.3 illustrates some examples of the topologies of various transporters.

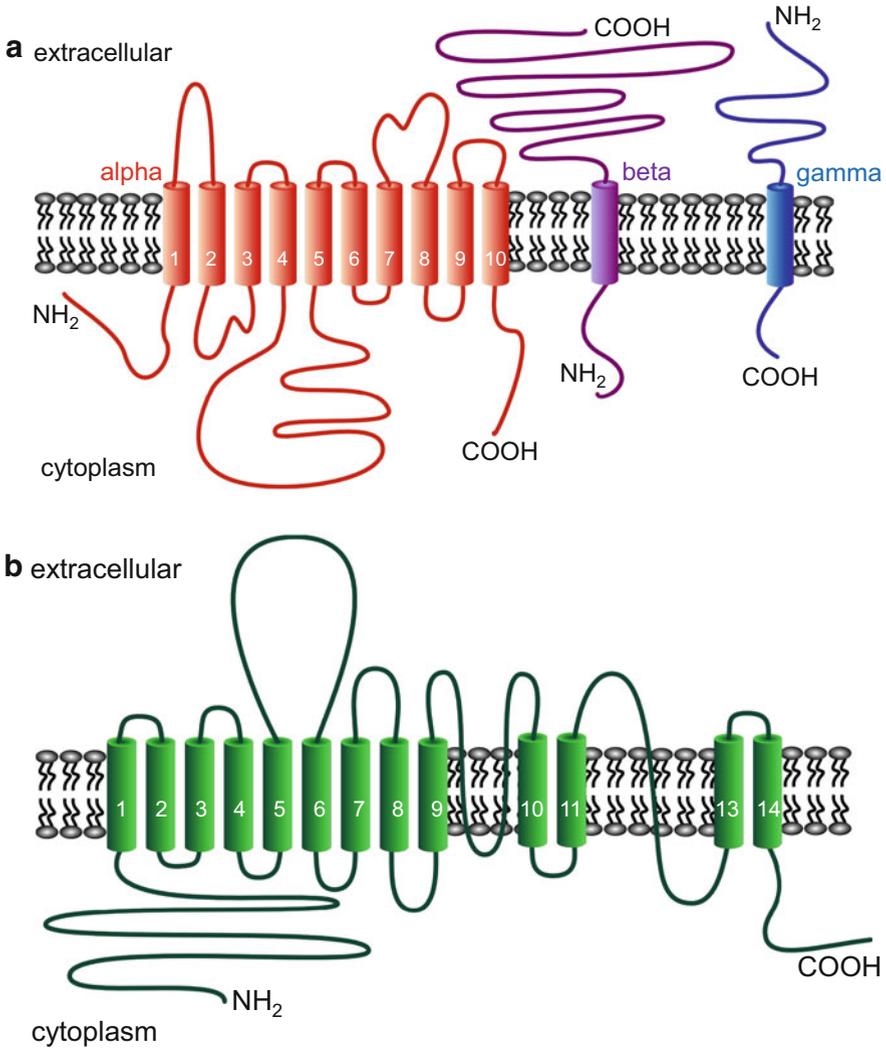


Fig. 2.3 (continued)

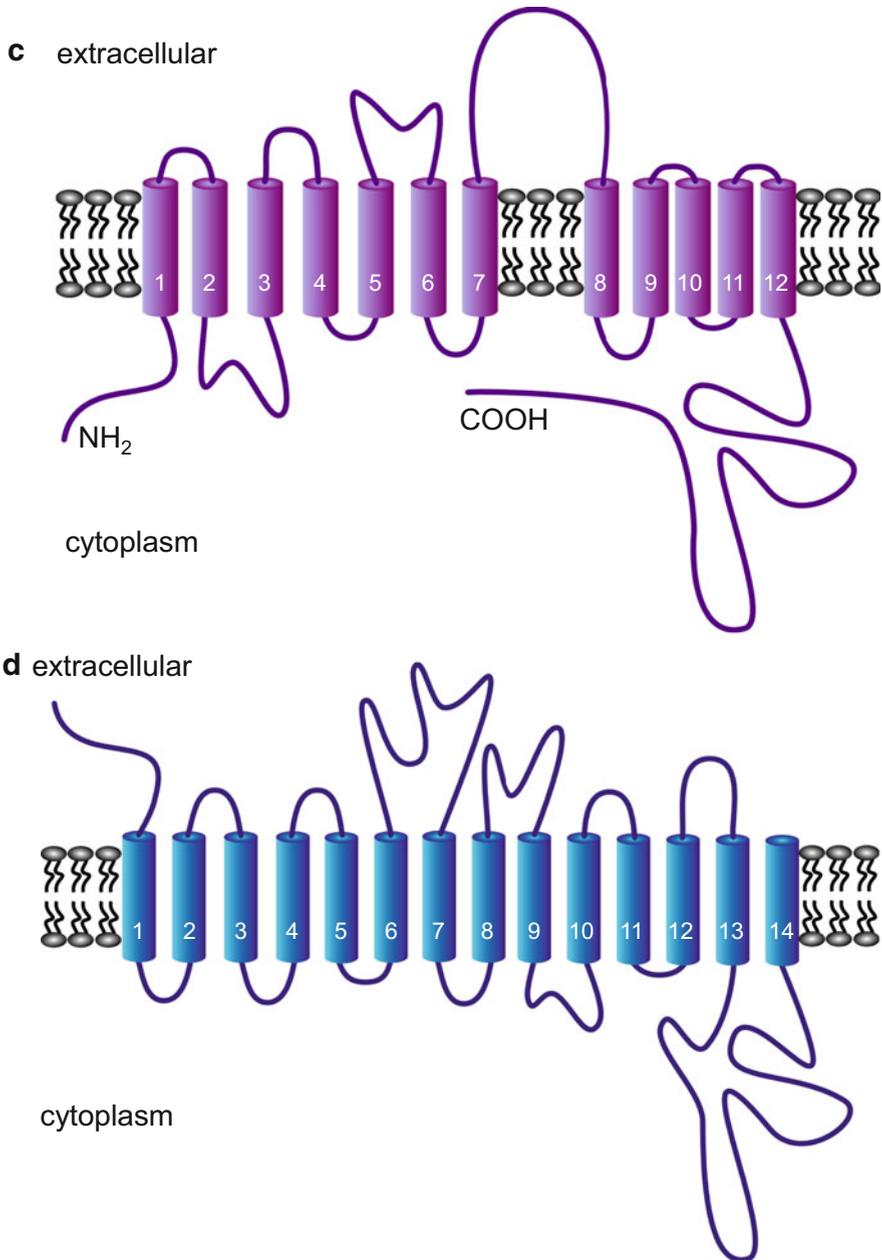


Fig. 2.3 Membrane topology of several Na^+ transporters involved in epithelial sodium absorption. (a) Organization of the α - and β -subunits of Na^+/K^+ -ATPases in the plasma membrane. The γ -subunit, which is found associated with some isoforms of Na^+/K^+ -ATPase, is also shown. (b) Presumed topology of the electrogenic $\text{Na}^+-\text{HCO}_3^-$ cotransporter. The model shows the extended cytosolic amino- and carboxy-terminal domains (NH_2 and C-termini) linked via a transmembrane

2.2.4.2 Passive Paracellular Transport

The paracellular pathway in renal tubular epithelia such as the PCT, which reabsorbs the largest fraction of filtered NaCl, is important for the transport of electrolytes and water (Krug et al. 2012; Muto et al. 2012). The gatekeeper of the paracellular pathway is the tight junction, which is located at apical cell–cell interactions of adjacent epithelial cells (see Fig. 2.1). The tight junction separates the apical domain from the basolateral domain and provides a barrier to paracellular movement of water and ions. Claudins are key integral proteins that provide the barrier function and permit selective paracellular transport (Yu 2015). Claudin-2 is the main claudin responsible for reabsorption of Na⁺ (Kiuchi-Saishin et al. 2002). In addition to the claudin family members, there are several other tight junction transmembrane proteins that might directly influence the adhesive barrier, including occludins, junctional adhesion molecules, and tricellulin (Anderson and Van Itallie 2009). Interestingly, approximately 30 % of the Na⁺ that is transported from lumen to blood by the transcellular pathway diffuses back to the urine by the paracellular pathway in the proximal tubule.

2.2.5 Methods of Na⁺ Transport Measurement

To a large degree, advancement in our understanding of the physiology of epithelial transport of sodium and other electrolytes has been afforded by the development of key measurement methods and tools. Stockand and colleagues have recently provided a clear overview and description of traditional and contemporary *in vivo* and *ex vivo* tools used to study renal tubule transport physiology (Stockand et al. 2012). These include isotopic flux measurements, micropuncture, and microperfusion techniques and the patch-clamp technique performed *ex vivo* in various configurations on isolated kidney tubules.

Fig. 2.3 (continued) domain that includes 14 transmembrane spans, one of which is thought to be an extended region rather than an α -helix [modified from Parker and Boron (2013)]. (c) The topology proposed for Na⁺-coupled chloride cotransporters (NKCC) [modified from Gamba (2005)]. (d) Secondary structure of Na⁺-glucose cotransporter SGLT. Shown are 14 transmembrane helices with both the NH₂ and COOH-termini facing the extracellular solution [modified from Turk et al. (1996), Wright et al. (2011)]

2.3 Sodium Homeostasis and Its Role in the Kidney

2.3.1 Role of Sodium Reabsorption in the Passive Diffusion of Water, Urea, and Other Solutes

Na^+ reabsorption is critical for the transport of other solutes and water homeostasis. When sodium is transported out of the kidney tubule, its concentration decreases inside the tubule while increasing in the interstitial space. Various active and passive transport processes are involved in the maintenance of water and solute homeostasis in different nephron segments. Shown in Fig. 2.4 are basic mechanisms by which water, chloride, and urea reabsorption are coupled with sodium transport in the kidney. The generation of transmembrane concentration differences of sodium and other solutes induces osmotic water flow from the lumen to the renal interstitium. Certain segments of the nephron, such as the proximal tubule, are highly permeable to water and small ions via the tight junctions in these segments. In addition, AQP1 was shown to be abundant in the apical and basolateral membranes of the proximal tubules and descending thin limbs of Henle's loop, where this water channel provides the transcellular pathway for water following small osmotic gradients at the apical and basolateral surfaces of the cell (Nielsen et al. 1993; Agre 2000). In contrast to the proximal tubules, less leaky epithelia such as the loop of Henle, distal tubules, and collecting duct prevent the osmotic flow of water across the tight junctions of the plasma membrane. However, vasopressin, also known as arginine vasopressin (AVP) or antidiuretic hormone (ADH), greatly increases water permeability in the collecting tubules, as discussed later (see Sect. 2.3.2.4). Therefore, changes in sodium reabsorption significantly influence the reabsorption of water and many other solutes.

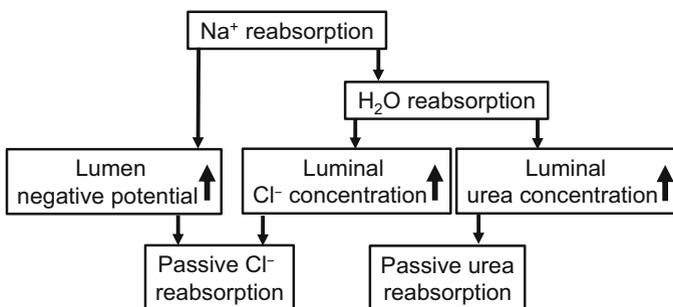


Fig. 2.4 Mechanisms of coupling of sodium and water, chloride, and urea reabsorption. When sodium is reabsorbed, anions such as chloride and bicarbonate are transported along with it; as Na^+ is positively charged, its transport from the lumen creates a lumen-negative potential, which promotes the diffusion of chloride through the paracellular pathway. Furthermore, transmembrane concentration differences of sodium facilitates water reabsorption from the lumen to the renal interstitium, which in turn can promote additional reabsorption of chloride ions and urea due to the increased concentration of these solutes present in the tubular lumen resulting from the water loss

In addition to water, sodium reabsorption plays an important role in the transport of other solutes. When sodium is reabsorbed across epithelial cells, anions such as chloride and bicarbonate are transported along with sodium through coupled transporters. As Na^+ is positively charged, its transport through channels from the lumen creates a lumen-negative potential, which promotes the diffusion of anions such as chloride through the paracellular pathway in certain nephron segments (e.g., PCT). Osmotic water flow from the lumen to the interstitium also promotes the additional reabsorption of chloride ions due to the increased chloride concentration present in the tubular lumen resulting from the water loss. Thus, sodium reabsorption is coupled to the passive reabsorption of chloride by changes in the electrical potential and chloride concentration gradient. Moreover, sodium and chloride transport are tightly linked since these ions can also be reabsorbed by secondary active transport through coupled cotransporters (e.g., NCC and NKCC, as discussed below).

Urea is mainly transported by UT-A and UT-B urea transporters (Klein et al. 2011, 2012). Besides, urea is also passively reabsorbed from the tubule. As described above, water is reabsorbed by osmosis from the lumen. Thus, urea concentration in the lumen increases, consequently, and urea is also reabsorbed by created concentration gradient. However, urea permeates the tubule to a much lesser extent than water or chloride. In addition to these facilitated urea transporters, sodium-dependent, secondary active urea transport mechanisms have been characterized. Thus, it was shown that removing sodium from the perfusate completely inhibited net urea reabsorption, demonstrating that active urea transport is dependent upon the presence of sodium in the tubule lumen (Isozaki et al. 1994; Sands et al. 1996). However, the active urea transporters have not yet been cloned (Klein et al. 2011).

2.3.2 Sodium Absorption in Different Nephron Segments

Precise regulation of Na^+ absorption by the kidneys relies on sequential actions of the various nephron segments, each with highly specialized transport capabilities. Each human kidney contains about one million nephrons capable of forming urine (Hoy et al. 2005). Figure 2.5 provides an overview of consecutive segments of the nephron and corresponding Na^+ transport proteins along the nephron. Every nephron is comprised of a renal corpuscle containing the glomerulus and Bowman's capsule, a proximal tubule (proximal convoluted and straight tubules), a loop of Henle, a distal convoluted tubule (DCT), connecting tubule (CNT), and the collecting duct system, which includes the initial collecting tubule (ICT), the cortical collecting duct (CCD), the outer medullary collecting duct (OMCD), and the inner medullary collecting duct (IMCD). All of the components of the nephron, including the collecting duct system, which collects urine from several nephrons, are functionally interconnected. In general, the absolute rates of Na^+ reabsorption are greatest in the proximal tubule and fall as the tubular fluid proceeds from proximal to distal segments. The proximal tubule reabsorbs the majority of the filtered Na^+ load (up

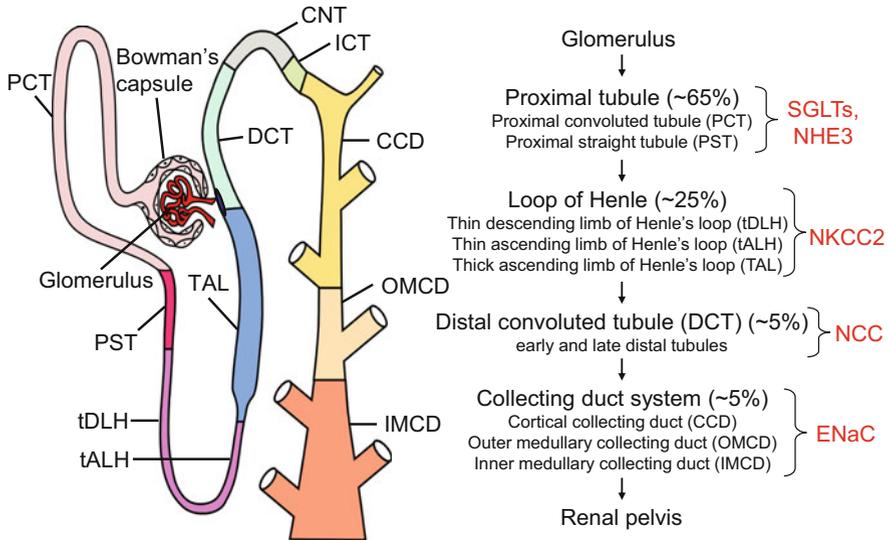


Fig. 2.5 Structure and consecutive segments of the nephron. Proximal convoluted tubule (PCT), proximal straight tubule (PST), thin descending limb of Henle's loop (tDLH), thin ascending limb of Henle's loop (tALH), thick ascending limb of Henle's loop (TAL), distal convoluted tubule (DCT), connecting tubule (CNT), initial collecting tubule (ICT), cortical collecting duct (CCD), outer medullary collecting duct (OMCD), and inner medullary collecting duct (IMCD) segments of nephron are shown. Approximate percentage of sodium reabsorption in different segments and major sodium transporters mediating these processes are also depicted [modified from Staruschenko (2012)]

to 60–70 %). However, as discussed below, the proximal tubule has a rather limited ability to alter Na⁺ transport. In contrast, the DCT and collecting ducts reabsorb only a minor fraction (approximately 5 %) of the filtered Na⁺, but are finely regulated by different physiological factors.

2.3.2.1 Proximal Tubule

The proximal tubule (PT) is the major site for Na⁺ absorption in the nephron. There is considerable heterogeneity of both morphologic and functional characteristics along the proximal tubule. Typically, the proximal tubule consists of convoluted and straight tubules (PCT and PST, respectively). Based on the ultrastructure, it can be further divided into S1, S2, and S3 segments. The first two segments are located in the kidney cortex, and the straight segments (S3) descend into the outer medulla. The net rates of the Na⁺ transport in the late proximal segment are, in general, lower than in the initial proximal convoluted tubule (Jacobson 1982). Importantly, the absorption of sodium in the proximal tubule provides the driving force for absorption of other solutes, such as bicarbonate, glucose, phosphate, and amino acids (Parker and Boron 2013; Skelton et al. 2010). Under most circumstances, fluid at

any given point along the proximal tubule has virtually the same Na^+ concentration and osmolality as plasma (Ullrich et al. 1963). The isosmotic nature of proximal tubule fluid absorption derives from the high water permeability of this segment (Andreoli et al. 1978), which effectively clamps the osmolality of the tubular fluid at that of plasma. Although Na^+ transport in the proximal tubule occurs in the absence of large electrical or chemical gradients, the bulk of Na^+ absorption in the proximal tubule involves active transport. Earlier studies demonstrated that Na^+ can be reabsorbed in this tubule segment against both concentration (Ullrich et al. 1963; Giebisch et al. 1964) and electrical (Barratt et al. 1974) gradients. A significant amount of Na^+ transport in the proximal tubules also occurs passively (Schafer et al. 1975). For example, in the late convoluted and straight proximal tubules, Na^+ diffuses passively out of the tubule driven by the lumen-positive electrical potential difference in those segments. This potential difference derives from a Cl^- concentration gradient across the tubule wall. Shown in Fig. 2.6 is a simplified scheme of Na^+ reabsorption in proximal tubules.

NHE3 NHE3 is a Na^+/H^+ exchanger involved in large amounts of neutral Na^+ absorption in the PCT. NHE3 (in human is encoded by the *SLC9A3* gene) is one of the nine isoforms of the mammalian Na^+/H^+ exchanger (NHE) gene family (Donowitz and Li 2007). NHE3 is localized at the apical plasma membrane of the proximal tubules and is responsible for the majority of sodium absorption in the kidney. NHE3 performs an electroneutral exchange of one extracellular Na^+ ion with one extracellular proton (see Fig. 2.2). NHE3 is functionally linked to a $\text{Cl}^-/\text{HCO}_3^-$ exchanger and takes part in neutral NaCl absorption (Soleimani 2013).

The activity of NHE3 is regulated allosterically by the intracellular pH, stimulated by cellular acidification. NHE3-mediated neutral NaCl absorption is highly regulated by dietary conditions and the endocrine environment under both physiological and pathophysiological conditions. NHE3 cycles between the apical plasma membrane and the recycling compartments under basal conditions. The mechanisms involved in acute regulation of NHE3 include changes in plasma membrane expression and turnover, which can be modulated by altering both endocytosis and exocytosis rates, and changes in the cellular half-life of NHE3 (for review, see Donowitz and Li (2007)).

SGLT There are two classes of glucose transporters involved in glucose homeostasis. Sodium–glucose cotransporters or symporters (SGLTs) are the active transporters driven by the inward Na^+ gradient maintained by the Na^+/K^+ pump. Transport of glucose via facilitated diffusion is mediated by GLUT transporters (uniporters). SGLTs are exquisitely selective for Na^+ as the cation required for cotransport with glucose. One interpretation of the cation selectivity data is that cation binding initiates a change in the conformation of the sugar binding site (Wright et al. 2011). Interestingly, SGLT1 (in humans is encoded by the *SLC5A1* gene) and SGLT3 (*SLC5A4*) have two strongly interacting Na^+ binding sites, while SGLT2 (*SLC5A2*) only has one Na^+ binding site (Díez-Sampedro et al. 2001; Hummel et al. 2011). As demonstrated in Fig. 2.2, SGLT1 has a 2:1 Na^+ to glucose transport stoichiometry.

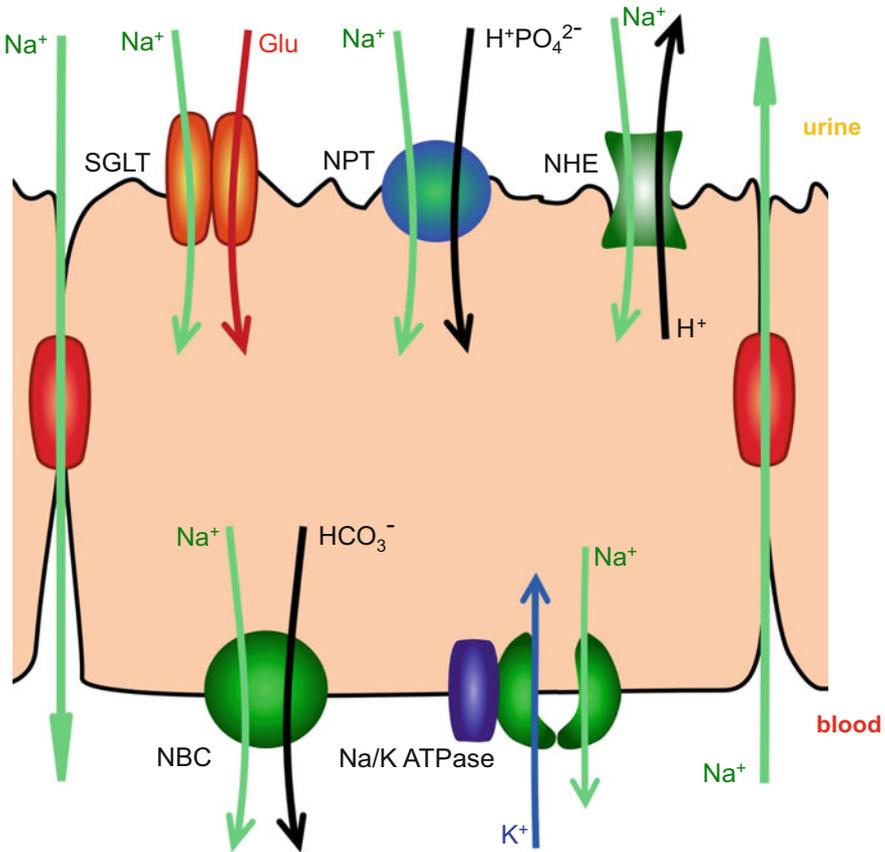


Fig. 2.6 Simplified scheme of Na^+ reabsorption in proximal tubules. In the proximal tubules, sodium can be reabsorbed against both concentration and electrical gradients; however, a significant amount of Na^+ transport also occurs passively. The majority of sodium ions are reabsorbed via the Na^+/H^+ exchanger (NHE); sodium–glucose cotransporter (SGLT) cotransports Na^+ and glucose into the cells, and so does the Na^+ –phosphate cotransporter (NPT) along with phosphate ions. Importantly, the absorption of sodium in the proximal tubule provides the driving force for absorption of other solutes, such as bicarbonate, glucose, phosphate, and amino acids. On the basolateral side, sodium is removed from the cells with the help of $\text{Na}^+/\text{HCO}_3^-$ cotransporter (NBC) and Na^+/K^+ -ATPase

SGLT1 cotransports Na^+ and glucose in the PCT. Activity of this transporter depolarizes the plasma membrane, which can serve as a signal for additional mechanisms. Interestingly, in the absence of glucose, SGLT1 can still conduct Na^+ currents. The first evidence that SGLT1 worked as uniporter was reported in 1990 (Umbach et al. 1990), soon after SGLT1 was cloned in 1987 (Hediger et al. 1987). Thus, it was shown that SGLT inhibitor phlorizin blocked a current in the absence of glucose. This current accounted for approximately 8 % of the total Na^+ –glucose cotransporter current and was not observed in control cells (Umbach et al.

1990). Moreover, this current was saturated with increasing external Na^+ concentration (Loo et al. 1999). In addition, SGLT1 transports water and urea along with Na^+ and glucose (Loo et al. 1999; Wright et al. 2011).

There are 12 members of the human SGLT (SLC5) gene family, including cotransporters for sugars, anions, vitamins, and fatty acids. However, only four members of the SLC5 family are Na^+ -glucose cotransporters. Two of these are responsible for glucose transport in the kidney: SGLT1 and SGLT2. SGLT3 is also expressed in the kidney. However, in contrast to SGLT1/2, it was demonstrated that SGLT3 functions as glucose sensor, not as Na^+ -glucose cotransporter (Díez-Sampedro et al. 2003). Early micropuncture studies identified that the glomerular filtrate is glucose-free when it reaches the end of the PCT. Further microperfusion experiments with isolated nephrons revealed that active glucose reabsorption occurs in the early segments of PCT via a low-affinity high-capacity system, whereas active reabsorption in the late PST occurred by a high-affinity, low-capacity system. As it was later identified, the majority of glucose (and Na^+ , respectively) is transported in the PCT by SGLT2 and the rest in PST by SGLT1 (Wright et al. 2011). However, while a critical role of SGLT2 has been demonstrated, the functional properties of SGLT2 in the kidney are less well studied than SGLT1 due to its poor expression in heterologous expression systems.

2.3.2.2 The Loop of Henle

The loop of Henle consists of structurally and functionally distinct thin limbs (thin ascending limb of Henle (tALH), thin descending limb of Henle (tDLH)) and the thick ascending limb (TAL) (refer to Fig. 2.5). Compared to the proximal tubule, the loop of Henle reabsorbs a smaller amount of filtered Na^+ (approximately 25 % of the total load (Bennett et al. 1968)). The key difference between the two segments is that Henle's loop taken as a whole reabsorbs more salt than water, whereas in the proximal tubule, this occurs in essentially equal proportions. Interestingly, the reabsorption of salt and water is performed in different places along the loop. The descending limb reabsorbs very little Na^+ or Cl^- , but it allows transport of water; both tALH and TAL are impermeable to water, but significantly reabsorb sodium and chloride (which is why they are often referred to as “diluting segments”). As a result, the fluid leaving the loop is hypo-osmotic relative to plasma, indicating that more salt than water has been reabsorbed. Ultimately, the loop of Henle is a nephron segment that allows water and salt excretion to be regulated independently.

Thin Ascending and Thin Descending Loops of Henle Intense water extraction via aquaporin water channels (Nielsen et al. 2002; Verkman 2006; Halperin et al. 2008) and near absence of sodium reabsorption in the tDLH concentrate tubular fluid (Kokko 1970; Kokko and Rector 1972). High interstitial levels of NaCl and urea provide additional osmotic energy for water reabsorption by tDLH. Therefore,

when the fluid exits the tDLH, it possesses a favorable gradient of luminal sodium, which facilitates further passive sodium reabsorption in the water-impermeable tALH (Imai and Kokko 1974). The formation of dilute urine by the loop of Henle begins at the tALH. Transepithelial movement of sodium from the lumen occurs via the paracellular pathway, whereas chloride diffuses transcellularly via Cl^- -K1 chloride channels, which are selectively localized to the tALH and appear to be the major mediators of chloride movement there (Reeves et al. 2001). At large, the osmolarity of the fluid leaving the tALH is decreased due to a fall in NaCl content, which occurs almost entirely via passive and paracellular pathways.

Thick Ascending Loop of Henle As the luminal fluid moves into the TAL tubules, the reabsorbing properties of the epithelium change. In contrast to the tDLH and tALH, the major mechanisms of sodium and chloride reabsorption in the TAL are active. As with the tALH, the TAL consists of a tight epithelium, which is impermeable to water (Hebert 1986).

As shown in Fig. 2.7, the dominant step of luminal sodium and chloride uptake in this segment occurs via the sodium–potassium–chloride cotransporter ($\text{Na}^+ - \text{K}^+ - 2\text{Cl}^-$; NKCC2 isoform; see below) (Greger 1981; Koenig et al. 1983; Markadieu and Delpire 2014). Cl^- -K1 (h Cl^- -Ka in human) localized at the basolateral membrane allows passive Cl^- transepithelial resorption in the TAL (Fahlke and Fischer 2010; Imbrici et al. 2014). An accessory Cl^- -K channel subunit, barttin, encoded by the gene product of *BSND*, is required to form functional channel (Birkenhager et al. 2001; Estevez et al. 2001). It was reported that Cl^- -K1 is upregulated in water-restricted animals (Fushimi et al. 1993; Uchida et al. 1993). Further studies revealed that the Cl^- -K1 knockout mice can maintain salt and water balance under normal conditions but are unable to concentrate urine under water restriction; deletion of Cl^- -K1 results in diabetes insipidus (Matsumura et al. 1999). The electroneutral process of $\text{Na}^+ - \text{K}^+ - 2\text{Cl}^-$ cotransport is driven largely by the favorable electrochemical gradient for Na^+ entry, which is maintained by the continuous operation of the Na^+/K^+ pump in the basolateral membrane. $\text{Na}^+ - \text{K}^+ - 2\text{Cl}^-$ transport is selectively inhibited by so-called loop diuretics (e.g., furosemide and bumetanide), as discussed further in Sect. 2.3.5.1 (Russell 2000). The apical membrane of TAL cells also expresses a Na^+/H^+ antiporter isoform (major isoform being NHE3 (Amemiya et al. 1995; Borensztein et al. 1995; Laghmani et al. 1997; Capasso et al. 2002)), which provides an alternative mechanism for the uptake of Na^+ and regulates intracellular pH.

Interestingly, NKCC2 requires equal amounts of Na^+ and K^+ to enter the cell. However, as there is much less K^+ than Na^+ in the lumen, the tubular fluid would seem to get depleted of K^+ long before enough Na^+ was reabsorbed. This issue is resolved by K^+ recycling via the apical K^+ -conductive pathway. K^+ leaks back into the lumen through apical potassium channels (mostly through ROMK channels, but involvement of other potassium channels has also been reported) (Guggino et al. 1987; Wang et al. 1990; Wang 1994, 2012; Giebisch 2001; Hebert et al. 2005), and is again available for $\text{Na}^+ - \text{K}^+ - 2\text{Cl}^-$ cotransport. Furthermore, K^+ channels actually

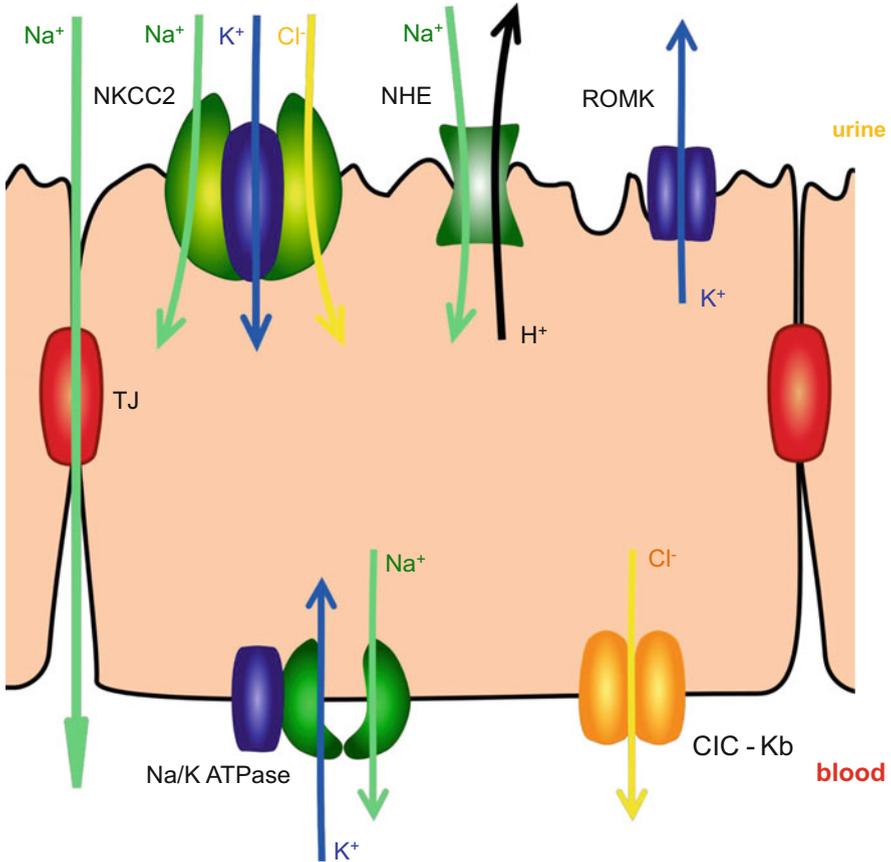


Fig. 2.7 Simplified scheme of Na⁺ reabsorption in the loop of Henle. Transepithelial movement of sodium from the lumen in the tDLH and tALH occurs via the paracellular pathway, whereas chloride diffuses transcellularly via CIC-K1 chloride channels. In contrast to the tDLH and tALH, the major mechanisms of sodium and chloride reabsorption in the TAL are active. The dominant step of luminal sodium and chloride uptake in this segment occurs via the sodium–potassium–chloride symporter (NKCC2 isoform); an alternative mechanism for the uptake of Na⁺ from the lumen is provided by the apical the Na⁺/H⁺ antiporter (NHE). CIC-K1 channels and Na⁺/K⁺-ATPase localized at the basolateral membrane allow Cl⁻ and Na⁺ transepithelial resorption, respectively. K⁺ enters the cell as a result of Na⁺/K⁺-ATPase function and then leaks back into the urine through apical potassium (ROMK) channels

dominate the apical membrane conductance of the TAL, creating a lumen-positive transepithelial voltage, which provides the driving force for the paracellular movement of Na⁺. This passive diffusion of Na⁺ accounts for as much as 50 % of total Na⁺ reabsorption in this segment. The Cl⁻ that enters TAL epithelial cells apically via NKCC2 exits via CIC-Kb Cl⁻ channels on the basolateral membrane (Fig. 2.7).

NKCC2 The uptake of Na⁺ in the loop of Henle is coupled to that of Cl⁻ and K⁺. The coupling ratio is fixed at 2 Cl⁻ per 1 Na⁺ and 1 K⁺ (see Fig. 2.2). The renal-specific apical form of this cotransporter encoded by the *SLC12A1* gene was denoted NKCC2 (also known as BSC1 or bumetanide-sensitive cotransporter), based on high homology with the basolateral Na⁺-K⁺-2Cl⁻ cotransporter NKCC1 (Gamba 2005). After its initial cloning from rat and rabbit (Gamba et al. 1994; Payne and Forbush 1994), NKCC2 was identified in the mouse exclusively in the kidney (Igarashi et al. 1995) and later in humans (Simon et al. 1996a).

NKCC2 belongs to a family of electroneutral cation-chloride cotransporters, which are all members of the amino acid polyamine cotransporter (APC) superfamily. As resolved from similarity to other crystallized APC family members, NKCC2 contains 12 transmembrane domains (see Fig. 2.3c) with a large extracellular loop between membrane segments 7 and 8; the *SLC12A1* gene encodes the protein with molecular weight of 115–120 kDa (approximately 1100 a.a.) (Gamba et al. 1994). There have been at least six different isoforms of NKCC2 identified in the mouse kidney (Payne and Forbush 1994; Igarashi et al. 1995; Yang et al. 1996; Simon et al. 1996a; Mount et al. 1999; Plata et al. 2001; Gamba 2001; Ares et al. 2011); such molecular diversity results from alternative splicing variants. Interestingly, NKCC2 isoforms are differentially expressed along the TAL. In situ RNA hybridization and RT-PCR studies have revealed that isoform A is expressed in the renal cortex and medulla outer stripe, F in the outer medulla with higher density in the inner stripe, and B in the renal cortex. In the macula densa, both isoforms A and B were found (Castrop and Schnermann 2008). Furthermore, A, B and F isoforms have different transport capabilities; A and B possess higher affinity to Na⁺, K⁺, and Cl⁻, than the F isoform (Plata et al. 2002).

The role of apical NKCC2-mediated Na⁺-K⁺-2Cl⁻ cotransport in the TAL was initially demonstrated by mutations in *SLC12A1*, which are associated with the Bartter syndrome (Simon et al. 1996a), a disorder characterized by hypokalemia, metabolic alkalosis, and hyperaldosteronism (see Table 2.1). NKCC2 knockout mice reproduce a clinical phenotype characteristic of this illness (clinical features included severe renal failure, high plasma potassium, and metabolic acidosis) (Takahashi et al. 2000). Furthermore, NKCC2 has been shown to play a role in the cardiovascular diseases, including hypertension. This is particularly important as this cotransporter is the main target of loop diuretics (e.g., furosemide and bumetanide), which are potent pharmacological agents currently in wide clinical use (Gamba 2005). Inhibition of NKCC2 results in reduced salt reabsorption in the TAL, which increases salt delivery to the distal nephron and produces substantial diuresis and natriuresis. It has been shown that NKCC2 along the TAL is activated in the Milan hypertensive rats, and this significantly contributes to the increase in systemic blood pressure in these rats (Carmosino et al. 2011). Several rare functionally impairing mutations in human NKCC2 were associated with lower blood pressure (Monette et al. 2011). In Dahl salt-sensitive (SS) rats, high salt intake increases apical NKCC2 expression and phosphorylation in the TAL as compared with salt-resistant animals, and this may contribute to enhanced NaCl reabsorption in SS rats during high salt intake (Haque et al. 2011; Ares et al. 2012). In general,

Table 2.1 Inherited disorders of renal sodium transport

Disorder	Affected gene product	OMIM #	Nephron distribution	Functional consequences	Clinical features
Hypertensive disorders					
Bartter, type 1 (antenatal)	NKCC2 (<i>SLC12A1</i>)	600839	TAL	Decreased NKCC cotransport	Hypotension, hypokalemia, metabolic alkalosis
Bartter, type 2 (antenatal)	ROMK (<i>KCNJ1</i>)	600359	TAL, CCD	Decreased apical K ⁺ recycling	Same as above
Bartter, type 3 (infantile)	ClC-Kb (<i>CLCNKB</i>)	602023	TAL	Decreased basolateral Cl ⁻ flux	Hypokalemia, alkalosis, hyperreninemia
Bartter, type 4	Barttin (<i>BSND</i>)	606412	tAL, TAL, inner ear	Decreased basolateral Cl ⁻ flux	As above, with sensorineural deafness
Bartter, type 5	CaSR (<i>CASR</i>)	601199	PT, TAL, parathyroid, thyroid, brain	Activating mutation of Ca ²⁺ -sensing receptor with decreased K ⁺ recycling and NaCl reabsorption	Hypokalemia, alkalosis, hyperreninemia, hypocalcemia
Gitelman	NCCT (<i>SCL12A3</i>)	600968	DCT	Decreased NaCl cotransport	Hypokalemia, metabolic alkalosis, hypocalciuria
Pseudohypoaldosteronism type 1 (autosomal recessive)	ENaC subunits (α , β , γ) (<i>SCNN1A</i> , <i>SCNN1B</i> , <i>SCNN1G</i>)	264350	Collecting duct	Decreased amiloride-sensitive sodium transport	Hypotension, hyperkalemia, metabolic acidosis
Pseudohypoaldosteronism type 1 (autosomal dominant)	Mineralocorticoid receptor (<i>NR3C2</i>)	177735	Collecting duct	Decreased response to mineralocorticoids	Hypotension, hyperkalemia (less severe than recessive)
Hypertensive disorders					
Liddle	β - or γ -ENaC (<i>SCNN1B</i> , <i>SCNN1G</i>)	177200	Collecting duct	Increased cell surface expression and activity of ENaC channels	Hypertension, hypokalemia, metabolic alkalosis, responsive to amiloride

Pseudohypaldosteronism type 2 (Gordon)	WNK1 or WNK4 (WNK1, WNK4)	145260	DCT, collecting duct	Increased NCC cotransport, increased paracellular Cl ⁻ permeability	Hypertension, hyperkalemia, metabolic acidosis, responsive to thiazide diuretics
Apparent mineralocorticoid excess	11β-HSD2 (HSD11B2)	218030	DCT, collecting duct	Decreased oxidation of glucocorticoids causing activation of mineralocorticoid receptor	Hypertension, hypokalemia, metabolic alkalosis, responsive to dexamethasone
Other disorders					
Renal glucosuria	SGLT2 (SLC5A2)	233100	Proximal tubule	Decreased sodium-coupled glucose absorption	Decreased threshold for glucosuria
Proximal RTA	NBC1 (SLC4A4)	603345	Proximal tubule	Decreased sodium-coupled HCO ₃ ⁻ transport across basolateral membrane	Metabolic acidosis, ocular and dental abnormalities, growth and mental retardation

Updated references available at <http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM>. Data provided are based on (Subramanya et al. 2013)
TAL, thick ascending limb, *tAL*, thin ascending limb, *DCT* distal convoluted tubule, *OMIM* # accession number for Online Mendelian Inheritance in Man.

NKCC2 constitutes the major salt transporting pathway in mammalian TAL, and its proper function is fundamental not only for salt reabsorption but for the kidney's ability to produce urine that is more dilute or concentrated than plasma, which is an essential for survival of the land mammals (Gamba 2005). Further details on structure and function of NKCC are provided in Chap. 12.

2.3.2.3 Distal Convuluted Tubule

The distal convoluted tubule (DCT) is the initial segment of the aldosterone-sensitive distal nephron (ASDN), which consists of DCT, the connecting tubule (CNT), and the collecting ducts (CD). DCT can be further divided into two functionally different segments, denominated “early” and “late” DCT (Reilly and Ellison 2000) by their sensitivity to aldosterone. Distal tubules reabsorb much smaller fractions of filtered Na^+ load than PCT or the loop of Henle (5–10 %) (Hierholzer and Wiederholt 1976), and this process occurs almost exclusively by the transcellular pathway. NaCl concentrations generated along the loop of Henle cannot be maintained by the DCT, and within the initial 20 % of the DCT, salt concentration almost doubles (Schnermann et al. 1982). Later luminal Na^+ concentration decreases to a value of approximately 30 mM at the end of DCT (Khuri et al. 1975). This occurs to a large extent via active Na^+ transport and because the transepithelial voltage becomes progressively more negative moving from the early to late DCT (Wright 1971; Barratt et al. 1975; Hayslett et al. 1977; Allen and Barratt 1985). The absorption of Na^+ in the DCT is dependent on the load. Higher tubular flow rates result in the increased delivery of Na^+ to the normally unsaturated later parts of the distal tubule (Khuri et al. 1975; Shimizu et al. 1989).

The major step of apical Na^+ intake in the early DCT is via the $\text{Na}^+\text{-Cl}^-$ cotransporter (NCC) (Velazquez et al. 1987), which is characteristically different from the NKCC2 in the TAL, independent of K^+ and highly sensitive to a different class of drugs—thiazide diuretics (see Sect. 2.3.5.2 and Fig. 2.8). As mentioned above, lumen transepithelial electrical potential is more negative in the late rather than early DCT, and this is probably caused by differences in the Na^+ transporters; the late DCT, as distinct from the early DCT, also has an amiloride-sensitive pathway of Na^+ reabsorption (Reilly and Ellison 2000), which is mediated by the epithelial Na^+ channel (ENaC; see Sect. 2.3.2.4) (Costanzo 1984; Ciampolillo et al. 1996; Schmitt et al. 1999; Loffing et al. 2000, 2001; Campean et al. 2001). However, species differences have been reported for ENaC expression in the late DCT. ENaC has been found in the DCT of mouse and rat renal tissues (Schmitt et al. 1999; Loffing et al. 2000) but not in human or rabbit kidney (Velazquez et al. 2001; Biner et al. 2002).

The removal of Na^+ from the basolateral side of the DCT cells is controlled by the very intense activity of the Na^+/K^+ pump in both early and late segments (Doucet 1988). Basolateral K^+ is then removed via the channels which belong to the K_{ir} family (Ookata et al. 2000; Lourdel et al. 2002; Hamilton and Devor 2012; Zhang et al. 2013; Zaika et al. 2013), likely Kir4.1 homomer (known as KCNJ10) or

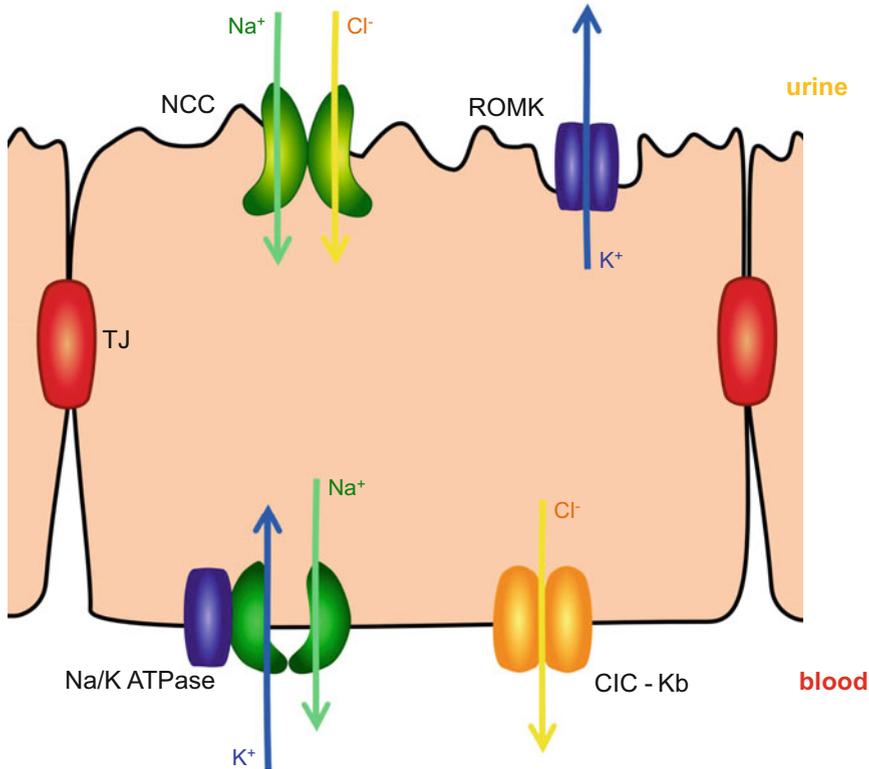


Fig. 2.8 Simplified scheme of Na^+ reabsorption in the distal convoluted tubules. The major step of apical Na^+ intake in the early DCT is via the Na^+-Cl^- cotransporter (NCC). The removal of Na^+ from the basolateral side of the DCT cells is controlled by the very intense activity of the Na^+/K^+ pump; apically, K^+ is secreted via ROMK channels. Similar to the TAL, basolateral Cl^- efflux is mediated via the CIC-K channels, which significantly ensure the maintenance of the gradient for the entry of Na^+ and mitigate against intracellular Cl^- concentration increases resulting from apical NaCl entry via Na^+-Cl^- cotransport

the Kir4.1/Kir5.1 (KCNJ10/16) heteromer, which help maintain the activity of the Na^+/K^+ pump and, thus, salt reabsorption capacity (Bockenhauer et al. 2009; Reichold et al. 2010; Paulais et al. 2011). Apically, K^+ is secreted via ROMK channels (Xu et al. 1997; Wang and Giebisch 2009; Wang et al. 2010). Similar to the TAL, basolateral Cl^- efflux is mediated via the CIC-K (mostly through CIC-K2/CIC-Kb) channels (Fahlke and Fischer 2010). These chloride channels significantly ensure the maintenance of the gradient for the entry of Na^+ and mitigate against intracellular Cl^- concentration increases resulting from apical NaCl entry via Na^+-Cl^- cotransport (Estevez et al. 2001; Lourdel et al. 2003). The apical membranes and tight junctions of DCT have almost no water permeability.

NCC The Na^+-Cl^- cotransporter (NCC, or *SLC12A3*) is a cation-coupled Cl^- cotransporter that, like NKCC2, belongs to the SLC12 family (Gamba 2005). NCC

is dose dependently inhibited by thiazide diuretics (e.g., chlorthalidone, hydrochlorothiazide, bendroflumethiazide, and metolazone) rather than loop diuretics (Stokes 1984; Costanzo 1985; Ellison et al. 1987; Gamba et al. 1994) and is thus also known as the thiazide-sensitive cotransporter (TSC). Moreover, it was also shown to be insensitive to barium, acetazolamide, furosemide, amiloride, 4,4'-diisothiocyanostilbene-2,2'-disulfonic acid (DIDS), and ouabain (Gamba 2005). NCC mediates coupled absorption of Na^+ and Cl^- ; this mechanism provides the means for maintaining intracellular Cl^- levels above the electrochemical equilibrium (Boron and Sackin 1983). Na^+ and Cl^- absorption in DCT is interdependent, and mammalian TSC has high affinity for both ions (half-maximal concentrations of both ions are ~ 10 mM) (Velazquez et al. 1984; Monroy et al. 2000).

The presence of NCC was first discovered in the urinary bladder of the winter flounder (Renfro 1975, 1977). Subsequent studies demonstrated that it is expressed in the renal cortex of rat, mouse, rabbit, and humans (Gamba et al. 1994; Mastroianni et al. 1996; Simon et al. 1996c; Bostanjoglo et al. 1998; Kunchaparty et al. 1999). The basic structure of NCC shares similarity with NKCC2 (shown in Fig. 2.3c), in that the protein has 12 transmembrane domains flanked by a short amino-terminal domain and a long predominantly hydrophilic C-terminal domain, both located in the cytoplasm; a long hydrophilic extracellular loop connects segments 7 and 8.

Loss-of-function mutations in the *SLC12A3* gene encoding NCC lead to the development of the Gitelman syndrome, which is clinically characterized by hypokalemia, hypomagnesemia, metabolic alkalosis, arterial hypotension, and hypocalciuria (see Table 2.1). On the other hand, increased NCC activity (caused by a gain-of-function mutation) results in the opposite symptoms including hyperkalemic metabolic acidosis, arterial hypertension, and hypercalciuria, known as Gordon syndrome, pseudohypoaldosteronism type II, or familial hyperkalemic hypertension (see Table 2.1) (Gamba 2005). New roles for NCC in sodium and potassium handling and blood pressure regulation have been unraveled. For instance, the recent discoveries that NCC is activated by angiotensin II but inhibited by dietary potassium shed light on how the kidney handles sodium during hypovolemia (accompanied with high angiotensin II) and hyperkalemia (Moes et al. 2014). In addition, much of the complex molecular machinery controlling the transporter's activity has been recently revealed. For example, modification of multiple kinases or ubiquitin ligases (including WNKs, SGK1, SPAK, Nedd4-2, Cullin-3, Kelch-like 3, and others), alteration of intracellular calcium signaling, changes in hormonal status (aldosterone, vasopressin, insulin, angiotensin II), and circadian rhythms all modulate the transporter's activity and may contribute to disease states such as hypo- or hypertension, and have detrimental effects on the ability of the kidneys to excrete sodium, potassium, calcium, and protons (Chiga et al. 2008; Vallon et al. 2009; Mutig et al. 2010; Gamba 2012; Chavez-Canales et al. 2013; Lee et al. 2013; Rieg et al. 2013; Saritas et al. 2013; Eladari et al. 2014; Gailly et al. 2014; Lagnaz et al. 2014; Moes et al. 2014; Richards et al. 2014; Terker et al. 2014). Further details on the structure, function, and regulation of NCC are provided in Chap. 13.

2.3.2.4 Connecting Tubule and Collecting Duct

Heterogenic vectorial transport in this segment is tightly controlled. This segment of the nephron is the most sensitive to hormones, which oversee the fine control of plasma Na⁺ and K⁺ concentrations. A variety of hormones (e.g., aldosterone, angiotensin II, vasopressin, atrial natriuretic peptide, and insulin) are involved in this tight regulation of CNT and CCD ion transport function. The late DCT, CNT, and CCD are often referred to as the aldosterone-sensitive distal nephron (ASDN). Aldosterone, which is the final element of the renin–angiotensin–aldosterone system (RAAS), increases the reabsorption of Na⁺ and water and the secretion of K⁺ ions.

The apical entry of Na⁺ in the CNT and the CCD is regulated by the epithelial Na⁺ channel (ENaC). Within the scope of this overview, only some aspects of ENaC regulation will be provided. Different aspects of ENaC regulation are highlighted in Chap. 19 and are subjects of several excellent recent reviews (Loffing and Korbmayer 2009; Bhalla and Hallows 2008; Butterworth et al. 2009; Soundararajan et al. 2010; Rossier 2014).

As described in Sect. 2.2.4.1, there are different transporters and exchangers involved in the maintenance of sodium homeostasis in the kidney. Interestingly, ENaC is the only “classical” channel mediating sodium reabsorption in the renal tubules. Considering that ENaC is responsible for the fine-tuning of sodium reabsorption in the last nephron segment, the role of this channel in sodium reabsorption in the kidney is critical and unique. While other transport proteins are also regulated by different factors, control of ENaC plays a key role in the final Na⁺ composition of urine. Evolutionarily, the ENaC/degnerin (Deg) superfamily members appeared in Metazoan ancestors (Studer et al. 2011), along with the Na⁺/K⁺ pump α - and β -subunits. The emergence of the ENaC/Deg superfamily and the complete form of the active Na⁺/K⁺ pump afforded more strict control of salt balance with changes in the environment. Like other genes involved in membrane transport and osmolarity regulation, the hypothesis that these genes helped regulate the transition to multicellularity is appealing. Evolution of ENaC and the Na⁺/K⁺ pump as limiting factors of aldosterone action on Na⁺ transport has been recently discussed by Rossier and colleagues (Studer et al. 2011).

Since ENaC is a channel that generally conducts Na⁺ ions in only one direction, it is possible to apply the patch-clamp electrophysiological approach to directly measure inward ENaC-mediated Na⁺ flux in native cells (Mironova et al. 2013; Stockand et al. 2012) and in overexpression mammalian systems (Staruschenko et al. 2006). Expression of ENaC subunits in *Xenopus* oocytes is also successfully used to assess ENaC function (Malik et al. 2005; Zhou et al. 2013; Chen et al. 2014; Krappitz et al. 2014). In combination with contemporary molecular genetics and biochemical tools, electrophysiology enables us to identify the function and specific mechanisms of action of renal ENaC channels. This has led to an explosive growth in investigation and subsequent understanding of many physiological and pathophysiological processes performed by ENaC in the aldosterone-sensitive distal

nephron. Single-channel data provide the most illustrative picture of channel-mediated Na^+ transport since transients from closed to open states represent flux of Na^+ ions through ENaC when channel is positioned in the open state. A more thorough description of ENaC structure, function, and regulation can be found in Chap. 19.

2.3.3 Physiological Regulation of Na^+ Absorption

The tight regulation of transcellular Na^+ concentrations is so important that multiple mechanisms work in concert to control them. The expression and activity of Na^+ channels and transporters are regulated by specific hormones and different extra- and intracellular regulatory mechanisms. Recently, significant progress has been made in our understanding of the cellular and molecular mechanisms responsible for Na^+ absorption in the kidney. Multiple laboratories have employed electrophysiological, biochemical, microscopical, molecular, and genetic methods to study ion channels and transporters mediating sodium transport in both normal and pathological conditions. The development and application of various tools to study proteins mediating renal function have revealed some of the intriguing physiological functions of the kidney. Research advances naturally resulted in the cloning of multiple genes that are involved in water and electrolyte transport in different renal tubular segments. Moreover, new knowledge about the function of particular segments of the kidney has been accrued as a result of the development and application of gene deletion techniques, both conventional and cell-specific gene knockouts (Rubera et al. 2009; Lang and Shumilina 2013; Wen et al. 2014).

Importantly, the same mechanisms controlling Na^+ absorption are implicated in the maintenance of other ions and solutes. Thus, multiple studies have demonstrated that the same mechanisms controlling activation of ENaC result in the downregulation of ROMK K^+ channels and vice versa (Frindt et al. 2011). Similar findings are reported for integrated control of sodium, chloride, bicarbonate, phosphate, and other ions. Recent studies have also revealed cross talk between ENaC and Na^+/K^+ pump that may play a role in the correlation between Na^+ delivery and reabsorption independently of hormonal influence (Wang et al. 2014).

2.3.4 Tubuloglomerular Feedback (TGF) Mechanisms

Changes in sodium reabsorption and glomerular filtration are closely coordinated to avoid fluctuations in urinary excretion. Autoregulation of renal blood flow and thus glomerular filtration rate (GFR) maintains constant delivery of ions etc to the distal nephron where fine control of their transport by hormones such as aldosterone and arginine vasopressin (AVP) governs water and electrolyte balance. The TGF mechanism operates at the single-nephron level to maintain distal delivery within

the narrow limits of the reabsorptive capacity of distal tubules (Braam et al. 1993). The mechanism for autoregulation during an acute increase in blood pressure is a reduction in afferent arteriolar radius that normalizes flow to the glomerulus. This arteriolar constriction is driven both by a myogenic mechanism and TGF, a response that senses NaCl delivery and transport by the apical NKCC cotransporter in the macula densa (MD) (McDonough et al. 2003). NKCC was proposed to be essential for the sensing step since intraluminal application of furosemide abolished the TGF-mediated reduction in single-nephron GFR (Wright and Schnermann 1974; Franco et al. 1988). MD is a region formed by about 20 cells in the tubular epithelium between the TAL and DCT where it establishes contact with its parent glomerulus and afferent arteriole. MD cell volume increases when increments are isosmotic and shrinks if osmolality increases. The MD cells are different from TAL and DCT epithelial cells both anatomically and functionally. For instance, in contrast to the water-impermeable TAL and DCT segments, the apical membrane of MD cells is permeable to water (Komlosi et al. 2006). Furthermore, MD cells have lower Na⁺/K⁺ pump activity than the adjacent tubular epithelial cells (Schnermann and Marver 1986; Komlosi et al. 2009).

TGF stabilizes nephron function through negative feedback by establishing an inverse relationship between the tubular NaCl load and the GFR of the same nephron. Although the molecular mechanisms of TGF signaling are still not completely uncovered, it is generally accepted that purinergic signaling via ATP or adenosine is at least partially responsible for the tubular signal-dependent regulation of glomerular hemodynamics (Braam et al. 1993; Peti-Peterdi 2006; Komlosi et al. 2009; Schnermann 2011). The sensor function is also coupled with other intracellular signaling events including changes in Cl⁻ concentrations, intracellular calcium, pH, membrane depolarization, cell volume, etc. Furthermore, in addition to purines, MD produces and releases prostaglandin E₂ and nitric oxide (Komlosi et al. 2009). MD also plays a critical role in renin secretion, which is the major component of the renin–angiotensin–aldosterone system (RAAS). Products of the RAAS formed downstream from renin act to conserve salt by causing glomerular vasoconstriction and by increasing reabsorption of salt and water from the proximal and distal nephrons.

In addition to the MD contacts with afferent arteriole, it was previously reported that the superficial nephrons of the renal cortex also come into close proximity to the corresponding afferent arteriole through the CNT (Barajas et al. 1986; Dorup et al. 1992; Capasso 2007). Following studies by Ren et al. provided evidence of the functional connection between the CNT and afferent arteriole (Ren et al. 2007). This cross talk was called “connecting tubule glomerular feedback” (CTGF) to differentiate it from TGF. Inhibition of ENaC with amiloride blocked the dilatation induced by CTGF, but inhibition of NCC with hydrochlorothiazide failed to prevent renal afferent arterioles dilatation (Ren et al. 2007). Thus, in addition to its critical role in collecting ducts, ENaC is important for sodium reabsorption in upstream CNT segment (Rubera et al. 2003; Nesterov et al. 2012). Recent studies provided some further details about specific mechanisms involved in CTGF (Ren et al. 2013, 2014; Wang et al. 2013). The CTGF response has not been widely studied and this

mechanism needs to be more fully explored. However, these studies emphasize the role of the connecting tubules and collecting ducts, where the final control of urinary electrolytes takes place.

2.3.5 Pharmacological Control of Na^+ Absorption

2.3.5.1 Loop Diuretics

As suggested earlier, the apical membranes of TAL epithelial cells are hyperpolarized because of high ROMK K^+ channel activity. Thus, the transmembrane potential of these apical membranes is strongly dependent on the equilibrium potential for K^+ (V_K). In contrast, the basolateral membrane has many Cl^- channels, so the basolateral transmembrane potential is less negative than V_K ; (i.e., the Cl^- conductance depolarizes the basolateral membrane). As a result of hyperpolarization of the luminal membrane and depolarization of the basolateral membrane, there exists a transepithelial potential difference of ~ 10 mV, with the lumen positive with respect to the interstitial space. The lumen-positive potential provides an important driving force for the paracellular flux of Na^+ , Ca^{2+} , and Mg^{2+} into the interstitial space. Inhibitors of $\text{Na}^+-\text{K}^+-2\text{Cl}^-$ cotransport thus also attenuate Ca^{2+} and Mg^{2+} reabsorption in the TAL by abolishing the transepithelial potential difference. Mutations in genes encoding the $\text{Na}^+-\text{K}^+-2\text{Cl}^-$ cotransporter, the apical K^+ channel, the basolateral Cl^- channel, the Barttin subunit of this Cl^- channel, or the calcium-sensing receptor on the basolateral membrane of these cells can all be causes of Bartter syndrome (inherited hypokalemic alkalosis with salt wasting and hypotension; see Table 2.1) (Simon et al. 1996a, b, c; Lifton et al. 2001).

$\text{Na}^+-\text{K}^+-2\text{Cl}^-$ cotransport inhibitors are chemically diverse. The drugs currently available in this group are furosemide and bumetanide, which contain a sulfonamide moiety; ethacrynic acid, which is a phenoxyacetic acid derivative; and torsemide, which is a sulfonylurea (Wargo and Banta 2009). These drugs inhibit NKCC2 at the apical membrane of the TAL cells of Henle's loop, so these diuretics are often called "loop diuretics." Diuretics acting at sites distal to the TAL have lower efficacy because a much smaller percentage of the filtered Na^+ reaches these distal sites. In contrast, NKCC inhibitors are highly efficacious because ~ 25 % of the filtered Na^+ normally is reabsorbed by the TAL and nephron segments distal to the TAL do not possess the reabsorptive capacity to rescue the flood of unreabsorbed salt from the TAL.

2.3.5.2 Thiazide Diuretics

Figure 2.8 illustrates the current model of electrolyte transport in the late DCT. As with other nephron segments, transport is powered by a Na^+/K^+ pump in the basolateral membrane. Energy in the electrochemical gradient for Na^+ is harnessed

by the NCC cotransporter in the luminal membrane and moves Cl⁻ into the epithelial cell against its electrochemical gradient. Cl⁻ then exits the basolateral membrane passively via a Cl⁻ channel. Thiazide diuretics inhibit the Na⁺-Cl⁻ cotransporter. Mutations in the Na⁺-Cl⁻ cotransporter cause a form of inherited hypokalemic alkalosis (Gitelman syndrome; see Table 2.1) (Gamba 2005, 2012).

Because the first inhibitors of the NCC were 1,2,4-benzothiazide-1,1-dioxides, this class of diuretics became known as thiazide diuretics. Later, drugs that are pharmacologically similar to thiazide diuretics, but are not chemically thiazides, were developed and are called thiazide-like diuretics. There are many thiazide and thiazide-like diuretics available in the USA, including hydrochlorothiazide, chlorothiazide, metolazone, and chlorthalidone. These drugs (especially hydrochlorothiazide) are often the first-line treatments for patients with hypertension. Moreover, because some resistance to the effects of high-dose loop diuretics may occur over time or in patients with chronic kidney disease or heart failure due to compensatory upregulation of DCT NCC expression and function, thiazide diuretics (esp. metolazone or chlorothiazide) are often used in this setting to potentiate diuresis in these patients (Fliser et al. 1994; Jentzer et al. 2010).

2.3.5.3 Amiloride and Its Analogues

Principal cells in the late DCT, CNT, and CD regulate ENaC expression in their apical membranes, which provides a conductive pathway for Na⁺ entry into the cell down the electrochemical gradient created by the basolateral Na⁺ pump (Staruschenko 2012). The higher permeability of the luminal membrane for Na⁺ depolarizes the luminal membrane but not the basolateral membrane, creating a lumen-negative transepithelial potential difference (TEPD). This transepithelial voltage provides an important driving force for the secretion of K⁺ into the lumen via K⁺ channels (primarily ROMK and the Ca²⁺-activated K⁺ channel (BK); see Fig. 2.9) in the luminal membrane. Loop and thiazide diuretics increase distal Na⁺ delivery, a situation that is often associated with increased K⁺ and H⁺ excretion. The increased luminal Na⁺ concentration in the distal nephron induced by such diuretics augments depolarization of the apical membrane and thereby enhances the lumen-negative TEPD, which facilitates K⁺ excretion. In addition to principal cells, the CD also contains type A intercalated cells (AI-Awqati 2013) that mediate H⁺ secretion into the tubular lumen via the apical vacuolar H⁺-ATPase (V-ATPase or proton pump), and this pump is aided by partial depolarization of the apical membrane. However, increased distal Na⁺ delivery is not the only mechanism by which diuretics increase K⁺ and H⁺ excretion. Activation of the renin-angiotensin-aldosterone system by diuretics also contributes to diuretic-induced K⁺ and H⁺ excretion, as discussed in the next section on mineralocorticoid antagonists.

Considerable evidence indicates that amiloride blocks ENaC in the luminal membrane of principal cells in the late distal tubule and CD (Warnock et al. 2014). Importantly, amiloride blocks ENaC in the low nM concentration range. Used in higher concentration, it is also able to inhibit other sodium channels and

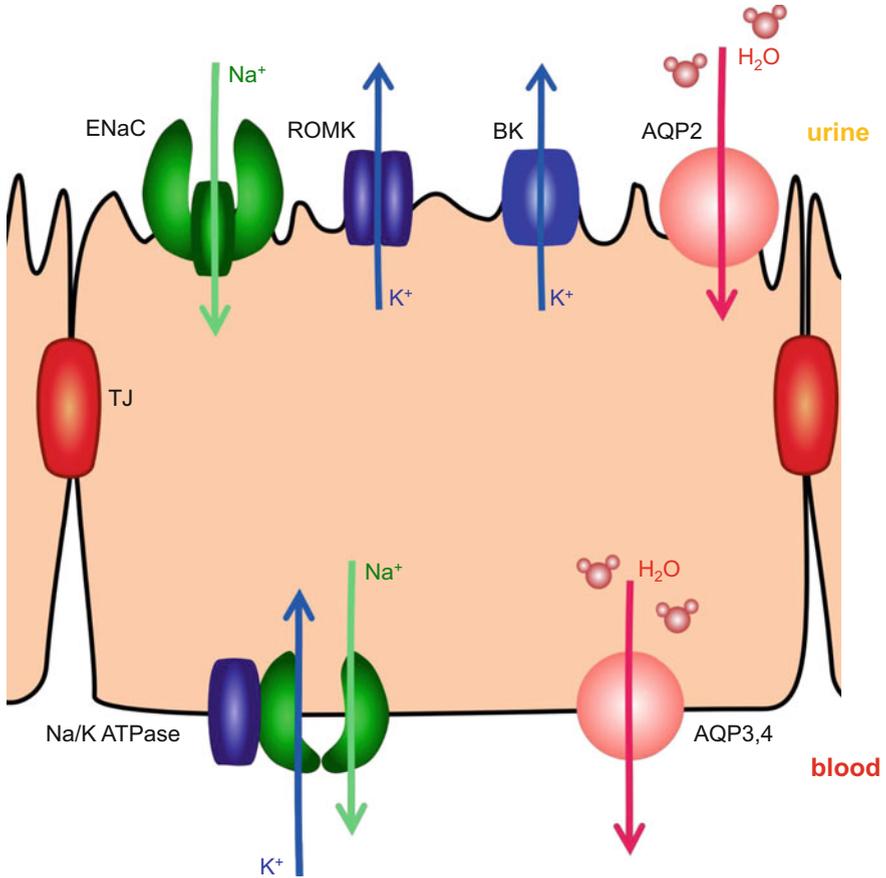


Fig. 2.9 Simplified scheme of Na⁺ reabsorption in the connecting tubules/cortical collecting duct. The apical entry of Na⁺ in the CNT and the CCD is regulated by the epithelial Na⁺ channel (ENaC); water follows the concentration gradient created by sodium transport and is taken up from the urine via AQP2 (aquaporin 2) channels. Basolaterally, sodium is reabsorbed via the Na⁺/K⁺-ATPase, and water is removed by aquaporins 3 and 4 (AQP3 and 4). Secretion of K⁺ into the lumen occurs primarily via ROMK and the Ca²⁺-activated K⁺ channel (BK)

transporters, such as NHE (Kleyman and Cragoe 1988; Teiwes and Toto 2007). Appropriate modification of amiloride has produced analogues that are several hundredfold more active than amiloride against specific transporters (Kleyman and Cragoe 1988). Liddle syndrome is an autosomal dominant form of low-renin, volume-expanded hypertension that results from C-terminal truncation mutations in the β - or γ -ENaC subunits, which prevents the channel's retrieval from the apical membrane and subsequent degradation, thus leading to increased basal ENaC expression and activity at the apical membrane (see Table 2.1) (Shimkets et al. 1994; Hansson et al. 1995a, b; Lifton et al. 2001; Ronzaud and Staub 2014).

Triamterene and amiloride are the only two drugs of this class in clinical use. Amiloride is a pyrazinoylguanidine derivative, and triamterene is a pteridine (Tamargo et al. 2014). Thus, both drugs are organic bases and are transported by the organic base secretory mechanism in the proximal tubule (McKinney 1984). Both drugs cause small increases in NaCl excretion and are often employed for their antikaliuretic actions to offset the effects of other diuretics that increase K⁺ excretion and may cause hypokalemia. Consequently, triamterene and amiloride, along with spironolactone (see next section), are often classified as potassium-sparing diuretics. These drugs are also often used in the setting of ongoing lithium treatment to limit the potential damage to CD epithelial cells caused by lithium entry via ENaC, which over time may result in nephrogenic diabetes insipidus (Kortenoeven et al. 2009; Kishore and Ecelbarger 2013).

2.3.5.4 Mineralocorticoid Receptor (MR) Antagonists

Mineralocorticoids induce salt and water retention and increase K⁺ and H⁺ excretion by binding to specific MRs. Currently, two MR antagonists are available in the USA: spironolactone and eplerenone. These MR antagonists are also called K⁺-sparing diuretics or aldosterone antagonists. Epithelial cells in the late distal tubule and CD contain cytosolic MRs that have high affinity for aldosterone. Aldosterone enters the epithelial cell from the bloodstream via the basolateral membrane and binds to MRs. The MR–aldosterone complex then translocates to the nucleus where it binds to specific hormone-responsive elements of DNA and thereby regulates the expression of multiple gene products called aldosterone-induced proteins (AIPs), which notably include the α -ENaC subunit, the serum and glucocorticoid-regulated kinase (SGK1) and the glucocorticoid-induced leucine zipper protein (GILZ). A number of different proposed effects of AIPs have been described, including increased cellular protein expression and localization of Na⁺ pumps and Na⁺ channels at the plasma membrane, changes in the permeability of tight junctions, and increased activity of enzymes in the mitochondria that are involved in ATP production (Law and Edelman 1978; Yu 2015). The net effect of AIPs is to increase Na⁺ conductance of the luminal membrane and sodium pump activity of the basolateral membrane. Consequently, transepithelial NaCl transport is enhanced, and the lumen-negative transepithelial voltage is increased. The latter effect increases the driving force for secretion of K⁺ and H⁺ into the tubular lumen.

2.3.5.5 SGLT Inhibitors

SGLT2 expressed in the PT has become one of the major targets for regulation blood glucose levels in diabetes (Oliva and Bakris 2014; De Nicola et al. 2014; Vallon et al. 2011, 2014). The classic competitive inhibitor of SGLTs is phlorizin (Ehrenkranz et al. 2005). Phlorizin has a higher affinity for hSGLT2 (K_i 40 nM) than hSGLT1 (200 nM). Several pharmaceutical companies have attempted to

modify the phlorizin structure to further enhance selectivity for SGLT2 over SGLT1 and develop oral SGLT2 inhibitors. Examples of these new FDA-approved SGLT2 inhibitors include dapagliflozin and canagliflozin (Riser Taylor and Harris 2013).

2.4 Sodium Balance and Its Role in Other Organs

2.4.1 Sodium Absorption in the Lung

Alveoli The alveoli of the lung promote the exchange of oxygen and CO₂ between the air spaces and the blood and contain two main cell types, termed alveolar type I and type II cells. Efficient gas exchange requires the presence of a thin liquid layer on apical surface of the alveolar epithelium, which is finely regulated by a balance between passive secretion of fluid from the vasculature by a paracellular route governed by permeability of claudins and active reabsorption governed by the presence of apical Na⁺ channels (reviewed in Eaton et al. (2009)). Although more is known about the surfactant-secreting type II cells, both alveolar cell types appear to contain a variety of ion transport proteins, including two forms of amiloride-sensitive channels. One form has the electrophysiological signature of ENaC expressed in the kidney and colon, as it is highly selective for Na⁺ over other cations and has a low single-channel conductance of ~5 pS and strong sensitivity to amiloride. The other form is relatively nonselective for Na⁺ over K⁺, has a higher single-channel conductance (~20 pS) and a weaker sensitivity to amiloride, and may contain primarily α -ENaC subunits rather than all three subunits. Na⁺ entering at the apical membrane exits at the basolateral membrane via the Na⁺/K⁺ pump. ENaC in the airways is highly regulated by agonists interacting with G protein-coupled receptors (e.g., purinergic and adrenergic agents), circulating hormones (e.g., glucocorticoids), chemokines and inflammatory mediators (e.g., TNF- α and interleukins), and reactive oxygen and nitrogen species. These regulatory factors may differentially modulate the function of highly selective ENaC channels versus nonselective cation channels (Eaton et al. 2009). Further details about the role of ENaC and other cation channels in the lung are provided in Chap. 19.

Airways Under normal conditions in the conducting airways, a balance is maintained between fluid reabsorption and fluid secretion to preserve airway surface liquid (ASL) height, cilia function, and thus normal mucociliary clearance. This balance appears to be regulated by ASL height itself and signaling factors such as secreted nucleotides, although the mechanisms that underlie this absorption–secretion coupling are still under active investigation. Apical membrane ENaC and possibly cyclic nucleotide-gated cation channels contribute to active transcellular Na⁺ reabsorption, which occurs in concert with basolateral Na⁺/K⁺ pump activity and paracellular Cl⁻ reabsorption (Hollenhorst et al. 2011). This active salt absorption is coupled with passive transcellular water flow mediated by aquaporin water

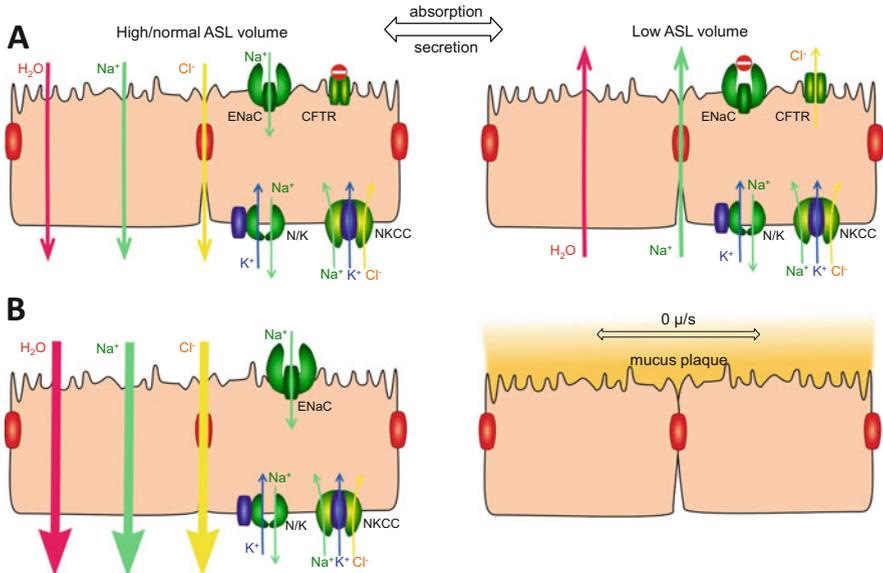


Fig. 2.10 Balance is maintained between airway surface liquid absorption and secretion mediated by transcellular and paracellular Na^+ , Cl^- , and water transport pathways under normal conditions (a), but this balance gets disturbed in patients with cystic fibrosis, who have excessive ENaC-mediated Na^+ (and thus fluid) absorption (b). See text for further details

channels (see Fig. 2.10) (Donaldson and Boucher 2003). On the other hand, fluid secretion in the conducting airways occurs via transcellular Cl^- secretion predominantly through apical CFTR channels, but also through Ca^{2+} -activated Cl^- channels like TMEM16A (Rock et al. 2009), coupled with basolateral NKCC-mediated Cl^- entry, paracellular Na^+ secretion (with ENaC inhibited), and transcellular water flow from the basolateral to apical compartment (Fig. 2.10).

Disease Correlations The importance of ENaC in the regulation of alveolar and airway surface liquid volume is evidenced by the fact that loss-of-function ENaC mutations underlie pseudohypoaldosteronism type I, which is associated with neonatal respiratory distress syndrome caused by pulmonary edema (Keszler and Sivasubramanian 1983; Malagon-Rogers 1999). Hypoxia decreases ENaC expression in nongenetic forms of this disorder among premature infants. In addition to inducing increased surfactant production, dexamethasone may improve this condition by counteracting hypoxia-induced decreases in ENaC-mediated sodium reabsorption. Decreased ENaC activity in the lung is also seen in patients susceptible to high-altitude pulmonary edema (Scherrer et al. 1999). A potential therapeutic role for ENaC modulation in the acute respiratory distress syndrome has also been explored (Matthay et al. 2005).

Perhaps the most thoroughly studied role for ENaC in pulmonary disease is in patients with cystic fibrosis (CF), caused by mutations in the CFTR Cl^- channel

(see Chap. 17). In patients with CF lung disease, the normal balance between fluid secretion and reabsorption in the airway gets disrupted due to a loss of CFTR function. As CFTR normally inhibits ENaC activity (by unclear mechanisms), a constitutive gain of ENaC functional activity appears to ensue in the airways of patients with CF, which might be a major contributing factor in the pathogenesis of CF airway disease, causing low ASL volume, mucous plugging, infections and inflammation, and chronic bronchiectasis with lung parenchymal destruction (Fig. 2.10) (Donaldson and Boucher 2007). Of note, a β -ENaC transgenic lung mouse model revealed that overexpression of ENaC appears to be sufficient to recapitulate the CF lung phenotype of decreased airway surface liquid volume and increased airway inflammation in mice (Mall et al. 2004). However, this view that low ASL volume, per se, underlies much of the pathogenesis of CF lung disease has been challenged, and the actual situation that exists in human CF lung disease is still far from settled (Song et al. 2009).

2.4.2 *Sodium Absorption in the Gastrointestinal and Endocrine Systems*

Small Intestine There are regional differences in the mechanisms of Na^+ transport and salt absorption vs. secretion in the gastrointestinal tract. Electroneutral NaCl absorption predominates in the small intestine and proximal colon and occurs at the apical membrane via parallel Na^+/H^+ exchange (mediated by NHE2 and NHE3) and $\text{Cl}^-/\text{HCO}_3^-$ exchange (Kato and Romero 2011). These transport proteins are regulated by intracellular pH and intracellular $[\text{HCO}_3^-]$ directly, as H^+ and HCO_3^- are substrates, and indirectly through pH-dependent allosteric effects. In addition, there is nutrient-coupled Na^+ absorption, which occurs via SGLTs for Na^+ -glucose cotransport, along with several Na^+ -amino acid cotransporters and Na^+ -coupled solute carriers. This salt and fluid absorption is balanced by electrogenic Cl^- secretion mediated by apical CFTR, which is stimulated under conditions that increase cellular cAMP, cGMP, or Ca^{2+} levels. These same signaling mediators concomitantly inhibit electroneutral NaCl transport. Once Na^+ and Cl^- are apically absorbed, the combination of a basolateral Cl^- channel (ClC-2), the Na^+/K^+ -ATPase, and the Kir7.1 K^+ channel, which allows K^+ pumped in by the Na^+/K^+ pump to recycle back out of the cell, affords the net transfer of NaCl across the basolateral membrane (Kato and Romero 2011).

Functional interactions among these transporters, along with a network of kinase signaling cascades, coordinate the regulation of salt absorption from the small intestine. The endocrine, autonomic, and immune systems can regulate epithelial NaCl transport function via the enteric nervous system and gene expression of the various transport proteins (Kato and Romero 2011). Glucocorticoids stimulate apical membrane NHE3 via SGK1 and also stimulate SGLT1 transporters (Grahammer et al. 2006). The mineralocorticoid aldosterone also weakly stimulates electroneutral NaCl absorption in the small intestine.

Colon In the colon there is electroneutral NaCl reabsorption under basal conditions, but aldosterone is a key stimulator of electroneutral NaCl absorption in the proximal colon and ENaC-mediated electrogenic Na⁺ reabsorption in the distal colon, where it works to enhance Na⁺ (salt) absorption from these segments of the gut (Harvey et al. 2008). Thus, in the distal colon, aldosterone causes a switch from electroneutral NaCl absorption to stimulated electrogenic Na⁺ absorption by inducing expression of apical ENaC and basolateral Na⁺/K⁺-ATPase (Kunzelmann and Mall 2002). In parallel, net K⁺ absorption is converted to net K⁺ secretion by induction of apical K⁺ channels (Sweiry and Binder 1989). ENaC expression at the apical membrane is generally more near the tips of the villi, whereas CFTR Cl⁻ channel expression is more pronounced at the apical membrane near the crypts of the villi. Like in the small intestine, CFTR is activated during secretion.

Disease Correlations Whereas enhanced apical Cl⁻ secretion via CFTR in the small intestine plays a key role in several secretory diarrheal diseases, such as cholera, where cholera toxin-induced cAMP formation promotes PKA-dependent CFTR-mediated fluid secretion, inhibition of sodium and chloride absorption in the colon is important for diarrheal disorders such as ulcerative colitis (Sandle 2005). Recent studies indicate reduced expression/activity of apical Na⁺ channels and basolateral Na⁺/K⁺-ATPase, leading to a loss of electrogenic Na⁺ absorption in the distal colon and rectum (Greig et al. 2004). There is also likely to be a decrease in electroneutral NaCl cotransport, which is present throughout the colon. Preliminary work on basolateral K⁺ channel abundance and activity in colonic epithelial cells suggests that whole-cell K⁺ conductance is decreased in ulcerative colitis, leading to epithelial cell depolarization and further limitation of Na⁺ absorption. In addition, there is a marked reduction in colonic epithelial resistance, which reflects a decrease in the integrity of intercellular tight junctions and the presence of apoptotic foci (Sandle 2005).

2.5 Sodium Transport in Epithelia and Human Diseases

In the kidney, the primary role of renal Na⁺ transport is the control of extracellular fluid volume. Thus, a number of genetic disorders are characterized by either hypo- or hypertension. Many Na⁺ transport proteins have been linked to specific genetic disorders in the kidney (Table 2.1). However, mutations in Na⁺ channels and transporters may cause disease manifestations in other organs such as lung and colon (described above) and in other tissues. Importantly, Na⁺ channels and transporters are highly critical for human diseases not only due to certain mutations identified in those proteins. Mutations in multiple genes causing human diseases are also involved in signaling mechanisms, where Na⁺ transport proteins are the final end points of those signaling pathways. Thus, ion channels and transporters might be attractive targets for various pharmaceutical drugs even when diseases are mediated by mutations in other proteins.

2.6 Final Conclusions

Epithelial Na⁺ reabsorption in the kidneys and other tissues plays key roles in regulating total body salt and water balance, airway function, and fluid and electrolyte reabsorption from the gut, glands, and other organs. In addition to focusing on individual Na⁺ channels and other transporters, future work will be required to provide us with detailed information about the integration between various components of sodium absorption and their tight regulation.

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