Proximal Tubule Function and Response to Acidosis

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Summary

The human kidneys produce approximately 160–170 L of ultrafiltrate per day. The proximal tubule contributes to fluid, electrolyte, and nutrient homeostasis by reabsorbing approximately 60%–70% of the water and NaCl, a greater proportion of the NaHCO3, and nearly all of the nutrients in the ultrafiltrate. The proximal tubule is also the site of active solute secretion, hormone production, and many of the metabolic functions of the kidney. This review discusses the transport of NaCl, NaHCO3, glucose, amino acids, and two clinically important anions, citrate and phosphate. NaCl and the accompanying water are reabsorbed in an isotonic fashion. The energy that drives this process is generated largely by the basolateral Na+/K+-ATPase, which creates an inward negative membrane potential and Na+-gradient. Various Na+-dependent countertransporters and cotransporters use the energy of this gradient to promote the uptake of HCO3- and various solutes, respectively. A Na+-dependent cotransporter mediates the movement of HCO3- across the basolateral membrane, whereas various Na+-independent passive transporters accomplish the export of various other solutes. To illustrate its homeostatic feat, the proximal tubule alters its metabolism and transport properties in response to metabolic acidosis. The uptake and catabolism of glutamine and citrate are increased during acidosis, whereas the recovery of phosphate from the ultrafiltrate is decreased. The increased catabolism of glutamine results in increased ammoniagenesis and gluconeogenesis. Excretion of the resulting ammonium ions facilitates the excretion of acid, whereas the combined pathways accomplish the net production of HCO3- ions that are added to the plasma to partially restore acid-base balance.


Introduction

The extracellular fluid (ECF) space provides a constant environment for the cells of a multicellular organism and prevents wide fluctuations in the ambient environment. This enables the cells to devote their gene products to more productive functions. The kidney is the principal organ that maintains the amount and composition of the ECF by executing functions of excretion, metabolism, and provision of endocrine substances. Most of these kidney functions occur in the proximal tubule, which is an ancient segment in mammalian nephron evolution.

In terms of excretion, the proximal tubule maintains an array of secretory mechanisms inherited from the more archaic secretory nephrons, which are ancestors of mammalian nephrons. The proximal tubule is also a tour de force of reabsorption of the glomerular filtrate. The filtration-reabsorption scheme is critical because, as metabolic rates escalated during mammalian evolution, GFR had to increase accordingly. The high GFR mandates a corresponding increase in reabsorption to prevent loss of valuable solutes and water. The proximal tubule fulfills most of the reabsorptive role for NaCl and NaHCO3, leaving the fine-tuning to the distal nephron. The proximal tubule also completes the reabsorption of glucose, amino acids, and important anions, including phosphate and citrate, because it is the sole site of transport of these filtered solutes.

In addition to solute reabsorption and secretion, the proximal tubule is also a metabolic organ. For example, within the proximal tubule, 25-hydroxy-vitamin D is converted to 1,25-dihydroxy-vitamin D, a hormone that increases blood Ca2+ levels. The proximal tubule is also the site of the 24-hydroxylase reaction that converts 25-hydroxy-vitamin D and 1,25-dihydroxy-vitamin D to their inactive forms (1). In addition, the proximal tubule is an important site of gluconeogenesis that parallels the liver (2). As an endocrine organ, the kidney also releases erythropoietin, renin, and Klotho into the systemic circulation and produces a plethora of locally active paracrine/autocrine and intracrine hormones, such as dopamine, endothelin, PGs, renin, angiotensin II, and so forth (1,3–5).

Space constraints do not permit a comprehensive account of proximal tubule function in this article. Thus, we will highlight NaCl and NaHCO3 handling as examples of reclamation of filtrate that are critical in preventing shock and fatal acidosis and where the proximal tubule accomplishes the bulk uptake, leaving the completion to the distal nephron. We will also briefly cover the reabsorption of glucose, amino acids, phosphate, and one organic anion, citrate, where the entire regulatory and absorptive function is confined to the proximal tubule. Whereas glucose and phosphate are primarily returned to the
circulation, citrate represents one substrate that is partially metabolized in the proximal tubule. Another organic substrate that is absorbed and metabolized is the amino acid glutamine. This process provides the nitrogen and carbon skeleton necessary to support renal gluconeogenesis and ammoniagenesis. Finally, the proximal tubule constantly adjusts its functions in response to needs, which is the hallmark of a stringent homeostatic system (6). Metabolic acidosis represents a state where there is concerted adaptations in multiple proximal tubule transport and metabolic functions aimed at minimizing the effect of the excess acid on the organism and rectification of the disturbance.

**NaCl** and **NaHCO₃ Transport**

Na⁺ is the primary cation that maintains the ECF volume (ECFV). Because Cl⁻ is four times more abundant than HCO₃⁻ as an ECFV anion, NaCl balance has become synonymous with ECFV regulation. NaHCO₃ is also a major ECFV solute, second only to NaCl, but it is the principal intracellular and extracellular buffer for H⁺. Thus, NaHCO₃ is better known for its role in acid-base balance than ECFV maintenance. There is limited regulation of gastrointestinal Na⁺, Cl⁻, or HCO₃⁻ absorption so the kidney is the primary organ that regulates extracellular electrolyte balance. The high GFR in humans (160–170 L/d) mandates reabsorption of the valuable filtered solutes. Otherwise, approximately 24,000 mmol of filtered Na⁺ and approximately 4000 mmol of filtered HCO₃⁻ would be lost per day with disastrous consequences. The proximal tubule is the first nephron segment after the glomerulus where reabsorption commences. It is important to note that proximal solute and water reabsorption proceeds primarily in an isotonic fashion with very small changes in luminal osmolarity. Figure 1A shows the profile of changes in selected solutes along the length of the proximal tubule. Figure 1B shows a generic cell model of how transepithelial transport is achieved. Transporters can broadly be viewed from a thermodynamic standpoint as being driven primarily by changes in enthalpy or entropy. Enthalpy-based or active transporters are directly coupled to ATP hydrolysis. They use energy released from the hydrolysis of phosphoanhydride bonds to move solutes uphill; hence, such transporters are by nature ATPases. Entropy-based or secondary active transporters dissipate existing electrochemical gradients to move a solute against a concentration gradient. Thus, they use the downhill free energy change of one solute to energize the uphill movement of another solute.

Transepithelial transport can occur via the paracellular or transcellular route, both of which are driven by electrochemical forces. The energy for solute movement is derived ultimately from high energy bonds in organic substrates taken up from the blood whose catabolism converts the energy into ATP (Figure 1B). Although there are multiple active transport systems directly coupled to ATP hydrolysis, the basolateral Na⁺/K⁺-ATPase is the principal consumer of ATP in the proximal tubule. It creates a low cellular [Na⁺] and negative voltage, which provides the

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**Figure 1.** General considerations of proximal tubule transport. (A) Profile of the tubular fluid to plasma ultrafiltrate ratio (TF/PUF). Selected solutes are shown along the length of the proximal tubule. PUF is a surrogate for the proto-urine in Bowman’s space. Inulin represents a filtered molecule that is neither secreted nor reabsorbed and the rise in its TF/PUF solely reflects reabsorption of water, which concentrates luminal inulin. Sodium reabsorption is near isotonic with water, which results in a very small increase in TF/PUF by the end of the proximal tubule. Inorganic phosphate (Pi) reabsorption is more rapid leading to rapid fall in TF/PUF. The fall in luminal [HCO₃⁻] is accompanied by a reciprocal rise in luminal [Cl⁻] as reabsorption remains by-and-large isotonic. Inorganic phosphate (Pi) reabsorption is more avid in the earlier parts of the proximal tubule. (B) Generic scheme of the proximal tubule cell. The primary energy currency is organic metabolic substrates that enter the proximal tubule and are catalyzed to produce ATP, which serves as the secondary energy currency. Some transporters are directly coupled to ATP hydrolysis (enthalpic transport), such as the H⁺/ATPase and Na⁺/K⁺-ATPase. The latter represents the main workhorse of the proximal tubule responsible for the majority of the cellular ATP consumption. The Na⁺/K⁺-ATPase converts the energy stored in ATP into low cellular [Na⁺] and high cellular [K⁺]. The presence of K⁺ conductance allows the [K⁺] gradient to increase the negative interior potential. The low cell [Na⁺] and negative voltage serve as the tertiary energy currencies that drive multiple secondary active apical transporters to achieve uphill movement of solutes coupled to downhill movement of Na⁺ (entropic transport). The transported solutes move in the same (symport or cotransport) or opposite (antiporn, exchanger, or countertransport) direction as Na⁺. Movement of solute can also proceed via paracellular routes driven by electrochemical forces.
ultimate energy to transport a multitude of solutes across the proximal tubule (Figure 1B). Apical secondary active solute entry can proceed through Na\(^+\)-dependent cotransporters (symporters), exchangers (antiporters), parallel transporter systems, or other Na\(^+\)-independent facilitated transporters (6).

**NaCl Transport**

Approximately 60%-70% of the filtered NaCl and the accompanying water are reabsorbed by the proximal tubule in a near isotonic fashion. This process is vital to the preservation of ECFV in the face of a high GFR. The foremost driving force is provided by the basolateral Na\(^+\)-K\(^+\)-ATPase, which sets the electrochemical gradient to drive a number of apical transporters that mediate Na\(^+\) and Cl\(^-\) entry (Figure 2) as bipartite-coupled parallel exchangers or tripartite-coupled parallel exchangers that all eventuate in net NaCl entry into the cell. Basolateral Cl\(^-\) exit is mediated by Cl\(^-\)-carrying exchangers and cotransporters (Figure 2) (7–9). Na\(^+\)-coupled transport is a general mechanism in the apical membrane so not all of the Na\(^+\) ions that enter the cell are devoted to NaCl transport (6). For example, a significant amount of glucose enters the apical membrane via Na\(^+\)-glucose cotransport (10). This electrogenic process (net positive charge moving into the cell) contributes to a slight negative luminal potential. In addition, the avid absorption of HCO\(_3^−\) in the early proximal tubule and the isotonic nature of the transport elevate the luminal [Cl\(^-\)] to above that of plasma (11). This combined electrochemical driving force in coalition with paracellular Cl\(^-\) permeability results in Cl\(^-\) movement from urine to blood, which is tantamount to essentially “Na\(^+\)-glucose-Cl\(^-\) absorption” (Figure 2). Alternatively, Na\(^+\) can leak back from the paracellular space into the lumen, providing a recycling system for Na\(^+\)-coupled transport of glucose.

The regulation of proximal tubule NaCl reabsorption facilitates the maintenance of ECFV. Hormones that maintain the ECFV and the integrity of the circulation through antinatriuresis stimulate proximal NaCl absorption. These include angiotensin II (12), endothelin (13), and \(\alpha\)-adrenergic stimuli (14). Conversely, natriuretic hormones, such as dopamine, inhibit proximal tubule NaCl reabsorption (15). Hormonal factors regulate largely transcellular NaCl flux via modulation of the Na\(^+\) transporters (16). Because NHE3, the apical Na\(^+\)/H\(^+\) exchanger, serves both NaCl and NaHCO\(_3^−\) reabsorption, it is not entirely clear how modulation of these two modes of transport can be dissociated when NHE3 is the prime target of regulation (17). In the proximal tubule, there is also a unique set of regulators that are largely physical in nature and involve coordinate coupling to regulation of GFR. These regulators are unlikely to operate in other nephron segments. When effective arterial blood volume is reduced, GFR is maintained by changes in arterial resistances that increase the filtration fraction despite lower renal plasma flow. This increases the protein concentration and oncotic pressure in the postglomerular blood, which along with the lower hydrostatic pressure jointly promote proximal fluid to move into the peritubular capillary.

**NaHCO\(_3^−\) Transport**

Whereas NaHCO\(_3^−\) contributes to the maintenance of ECFV, HCO\(_3^−\) is one of the major buffers that protect an organism from constant and pervasive acidification. A 70-kg human contains a free [H\(^+\)] of 40 nM in about 42 L of water. Consumption of a high-protein Western diet results in a net production of 50–70 mEq of H\(^+\) per day. Thus, in the absence of an appropriate buffer, the daily production of H\(^+\) will decrease the body pH to <3 within an hour, which is clearly not compatible with life. HCO\(_3^−\) ions provide the major buffer system that prevents the rapid acidification of the ECF. The kidney is the primary organ that controls plasma [HCO\(_3^−\)]. The burden of renal transport is to excrete an amount of acid equivalent to the daily net H\(^+\) production plus the amount of filtered HCO\(_3^−\), which is equivalent to the addition of acid if not reclaimed. HCO\(_3^−\) reclamation is achieved not so much by HCO\(_3^−\) reabsorption but, rather, by H\(^+\) secretion. The approximately 4000 mEq/d of HCO\(_3^−\) reclaimed is much higher than any daily dietary acid or base burden. Whether the organism is consuming a net acid diet of 50 mEq H\(^+\) equivalent per day versus a net alkaline diet of 50 mEq OH\(^−\) equivalent per day, the H\(^+\) that needs to be secreted into the lumen is 4050 mEq/d versus 3950 mEq/d, respectively. Therefore, the proximal tubular epithelium is
perpetually engaged in an H⁺-secreting mode regardless of the dietary load.

The proximal tubule plays a pivotal role in many aspects of acid-base homeostasis, which extends beyond HCO₃⁻ reclamation and H⁺ excretion (18). Because the urine cannot possibly maintain a pH low enough to hold 50–70 mEq of free H⁺, urinary buffers carry the majority of the H⁺. The proximal tubule synthesizes the most important open buffer (NH₃/NH₄⁺) and regulates the most abundant closed buffer (HPO₄²⁻/H₂PO₄⁻), which will be discussed in more detail below. Finally, the most abundant base equivalent in urine is citrate²⁻/³⁻, whose urinary excretion is also exclusively regulated by the proximal tubule (19).

The proximal tubule fulfills the roles of reclamation of HCO₃⁻ and secretion of H⁺ through the uphill transport of H⁺ into the lumen, a process collectively referred to as renal acidification. Classic disequilibrium pH experiments have detected very little direct HCO₃⁻ reabsorption (20). In the proximal tubule, H⁺ is secreted into the lumen mostly by electroneutral Na⁺/H⁺ exchange. The active transport of H⁺ by the H⁺-ATPase (V-ATPase) also contributes, but to a lesser extent (Figure 3) (21,22). The main Na⁺/H⁺ exchanger isoform is NHE3 but NHE8 also participates, particularly in the neonate where NHE3 is not fully expressed (23,24). In addition to H⁺, NHE3 also mediates the secretion of NH₄⁺ formed in the cell by titration.
of NH₃, which is tantamount to net H⁺ secretion (25). The H⁺ exported into the lumen has multiple fates (Figure 3A). It combines with the filtered HCO₃⁻ and, under the action of luminal carbonic anhydrase (CA-IV), generates CO₂, which diffuses into the cell and reconstitutes H⁺ and HCO₃⁻. The combined reaction accomplishes the reclamation of filtered HCO₃⁻ (Figure 3A). The secreted H⁺ also titrates citrate from its trivalent form into its divalent form, which is the preferred substrate for uptake by the Na⁺ dicarboxylate cotransporter, NaDC-1 (Figure 3B) (26). The reabsorption of citrate²⁻/³⁻ is equivalent to reabsorption of alkali (27). Finally, H⁺ secretion also titrates divalent HPO₄²⁻ to monovalent H₂PO₄⁻ that is not transported by NaPi-2a and NaPi-2c (Figure 3B), leading to phosphaturia and increased titratable acid or nonammonia urinary buffer carrying H⁺ ions in the urine. The HCO₃⁻ generated intracellularly by apical H⁺ secretion or ammoniagenesis exits the basolateral membrane via the family of Na⁺-bicarbonate cotransporters (28). Notably, the highly electronic NBCe1A (NBC1, SLC4A4), which is a splice variant of the electrogenic family of NBCe1, is responsible for basolateral HCO₃⁻ exit (29).

Approximately 70–90% of the filtered HCO₃⁻ is reabsorbed by the proximal tubule. Thus, this segment plays a pivotal role in the reclamation of HCO₃⁻. In terms of net H⁺ excretion, all of the NH₃/NH₄⁺ in the final urine is synthesized in the proximal tubule. The luminal pH at the end of the proximal straight tubule is approximately 6.7–6.8, which means virtually all of the NH₃ is titrated to NH₄⁺ (pK = 9.3). Likewise, half of the phosphate is already in its monovalent form. Therefore, the proximal tubule plays an essential role in net acid excretion because a large fraction of the urinary buffers is already titrated by the end of the proximal tubule.

**Other Solute Transport**

Another primary function of the proximal tubule is the recovery of metabolites from the glomerular filtrate. Approximately 180 g of glucose (1000 mEq) and 50 g of amino acids (400 mEq) are filtered by the human kidney per day. The process of transepithelial transport normally accomplishes the recovery of 99.8% of these metabolites from the luminal fluid of the proximal tubule. As mentioned previously, this process requires the asymmetric association of distinct transporters in the apical and basolateral membranes. Typically, a secondary active Na⁺-dependent transporter in the brush border membrane uses the Na⁺ gradient to accomplish the initial uptake of solutes. By contrast, the subsequent transport of the solutes across the basolateral membrane frequently utilizes a Na⁺-independent passive transporter. Important examples of transepithelial transport are those involved in the recovery of glucose, glutamine, citrate, and phosphate.

**Glucose Transport**

Two distinct Na⁺-dependent transporters mediate the uptake of glucose from the lumen of the proximal tubule (30). SGLT2 (SLC5A2) is a moderate affinity glucose transporter that mediates the cotransport of glucose and one Na⁺ ion. The SGLT2 transporter is localized to the brush border membrane of the S1 and S2 segments of the proximal tubule where it extracts the bulk of the filtered glucose. By contrast, SGLT1 (SLC5A1) has a slightly greater affinity, cotransports two Na⁺ ions per glucose, and is preferentially expressed in the apical membrane of the S3 segment. The cotransport of two Na⁺ ions makes the uptake of glucose energetically more favorable and thereby increases the concentrative power of the SGLT1. Thus, the sequential positioning of the two transporters that favor capacity and affinity in the early and late tubule, respectively, creates an effective mechanism to ensure that <1% of the filtered glucose exits the proximal tubule. Selective knockout studies indicate that SGLT2 normally accounts for 97% of the net glucose reabsorption, whereas SGLT1 removes the residual glucose and provides reserve capacity (31). Selective inhibition of SGLT2 has been proposed as a potential therapy for treatment of diabetes that may ameliorate glycemia and reduce the associated hyperfiltration (32,33). The basolateral membranes of the early and late segments of the proximal tubule contain the Na⁺-independent glucose transporters, GLUT2 and GLUT1, respectively. Both transporters facilitate the passive movement of glucose from the proximal tubular cells to the interstitial space. The proximal tubule metabolizes little or no glucose, which is compatible with the very low hexokinase in this segment (34). In normal acid-base balance, the arterial-venous difference for glucose across the kidney is zero or slightly positive, which reflects substantial proximal tubule gluconeogenesis counterbalanced by glucose consumption by the rest of the nephron segments (35).

**Amino Acid Transport**

The renal transport of amino acids is a complex process due to the range of structures and ionic properties of the free amino acids in the plasma (36,37). However, >80% of the filtered amino acids are neutral amino acids, all of which are recovered by the apical B’AT1 (SLC6A19) transporter. B’AT1 is a broad specificity Na⁺-dependent cotransporter that is expressed in the early portion of the proximal tubule and that binds various neutral amino acids, including glutamine, with relatively low affinities (38). Previous micropuncture studies established that the filtered glutamine is nearly quantitatively reabsorbed from the lumen of the early proximal tubule (39). Mutations in the B’AT1 transporter result in Hartnup disorder, which is characterized by a pronounced urinary loss of neutral amino acids (40,41). A separate Na⁺- and H⁺-dependent cotransporter (SLC1A1) recovers the acidic amino acids (42), whereas an antiporter (SLC7A9) mediates the uptake of basic amino acids and cysteine in exchange for a neutral amino acid (37). The transporters that mediate the export of amino acids across the basolateral membrane are less well characterized. However, it is thought that in normal acid-base balance, LAT2-4F2hc (SLC7A8), a heterodimeric obligatory neutral amino acid exchanger, mediates the efflux of glutamine from the proximal tubule (36,43). A related heterodimeric exchanger, y’LAT1-4F2hc (SLC7A7), promotes the export of basic amino acids in exchange for a neutral amino acid (44). The two antiporters contribute to the maintenance of normal intracellular levels of amino acids. However, net efflux requires the participation of a uniporter that facilitates the passive export of neutral
amino acids. The TAT1 transporter (SLC16A10) may accomplish the latter process (37).

**Organic Cation and Anion Transport**

The proximal tubule handles a very broad range of organic cations and anions that utilizes a variety of transporters operating in absorptive and secretory modes (45); however, due to space limitations, we will focus our discussion on citrate. The reabsorption of filtered citrate occurs in the proximal tubule apical membrane by NaDC-1 (SLC13A2), a Na⁺-dependent dicarboxylic acid cotransporter (46). The preferred substrates are dicarboxylates, such as citrate, succinate, fumarate, and α-ketoglutarate. In the proximal tubular lumen, citrate exists in equilibrium between its divalent and trivalent forms but citrate²⁻ is the transported species. Once it is absorbed from the lumen, citrate can be metabolized by cytoplasmic ATP citrate lyase to oxaloacetate and acetyl-CoA or shuttled into the mitochondria where it enters the citric acid cycle (47). When citrate²⁻/³⁻ is converted to CO₂ and H₂O, 2 or 3 H⁺ ions are consumed. Therefore, each milliequivalent of citrate excreted in the urine is tantamount to 2 or 3 OH⁻ lost. In the normal day-to-day setting in a human on a Western diet, there is a negligible amount of HCO₃⁻ in the urine. Citrate is the only organic anion in millimolar quantities in the urine and represents the main mode of base excretion under normal circumstances. In the face of large alkali loads, bicarbonaturia becomes the main mechanism of base excretion.

Citrate serves a dual purpose in the urine. As indicated above, it is a urinary base. In addition to base excretion, the 1:1 Ca²⁺:citrate³⁻ complex has a very high association constant and solubility. These properties render citrate the most effective chelator of calcium in the urine, which prevents its precipitation with phosphate and oxalate (48,49). Hypocitraturia is a major underlying cause of human kidney stones and, thus, citrate is the most important urinary anion for clinicians to understand (49).

**Phosphate Transport**

Phosphate homeostasis at the whole-organism level involves coordinated fluxes in the intestine, bone, and kidney, and endocrine cross-talk constituting a complex multiorgan network (50). Although both the intestine and bone are critical organs for phosphate homeostasis, this manuscript will only discuss the renal component. It is important to state that intestinal phosphate absorption involves significant paracellular uptake that is poorly regulated (51). Thus, the kidney is the paramount regulator of external balance. Unlike Na⁺ reabsorption where the finishing fine-tunings are achieved by more distal segments, phosphate reabsorption is accomplished almost entirely by the proximal tubule (52). A small contribution from the distal tubule has been proposed, but is still disputed (53). Plasma levels reflect total body phosphate status but are very insensitive; spot urinary concentrations are confounded by water excretion rate; and excretion rates of phosphate are affected by ingestion. Thus, all of these parameters are less than ideal to evaluate renal tubular phosphate handling. The parameters listed in Figure 4A are better suited to probe the proximal tubular handling independent of the excreted or filtered load.

The cellular model for proximal tubule phosphate transport is shown in Figure 4B. The primary driving force is the

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**Figure 4.** Proximal tubule phosphate transport. (A) Concepts of renal inorganic phosphate (Pᵢ) homeostasis by the proximal tubule. The flux of filtered and reabsorbed Pᵢ is plotted against plasma phosphate concentration; the difference between the two yields the rate of excretion of Pᵢ. There are a number of terms used to quantify the proximal tubule’s Pᵢ reabsorption at the whole organism level. Fractional excretion of Pᵢ (FEP) and tubular reabsorption of Pᵢ (TRP) sum to unity (FEP=1−TRP). The maximal tubular reabsorptive capacity of Pᵢ (TmP in units of mass/time) refers to the saturating transepithelial flux of Pᵢ, that the tubule can mount and is equal to the difference between filtered and absorbed phosphate when the filtered load is higher than TmP. The plasma concentration threshold at which Pᵢ starts to appear in the urine is TmP/GFR (in units of mass/volume). (B) Cell model of proximal tubule Pᵢ transport. Three apical transporters mediate Pᵢ entry with different preferred valence of Pᵢ, stoichiometry of Na⁺, electronegativity, and pH gating. The affinities for Na⁺ are all approximately 30–50 mM but are much higher for phosphate (0.1, 0.07, and 0.025 mM for NaPᵢ-Ila, NaPᵢ-IIc, and PIT-2, respectively). Distribution in the proximal tubule segments (S1, S2, S3). Basolateral Pᵢ exit occurs via unknown mechanisms. Apical Na⁺-coupled Pᵢ transport is inhibited in acidosis by alteration in luminal substrate, directly gating of the transporter by pH, and decreased apical NaPᵢ transporters as described in Figure 3B.
Na⁺-K⁺-ATPase generating an electrochemical gradient for apical phosphate entry. The current model predicts three transporters for apical entry; NaPi-2a (Npt2a, SLC34A1), NaPi-2c (Npt2c, SLC34A2), and Pit-2 (Npt3, SLC20A2, Ram-1). The disparate properties of the three transporters were reviewed in great detail by Virkki and colleagues (54). At present, the basolateral mechanism of transporters were reviewed in great detail by Virkki and colleagues (54). At present, the basolateral phosphate exit still remains enigmatic. It is possible that one or more of the plethora of anion exchange mechanisms may mediate phosphate exit. Thus far, there is little evidence for regulation of basolateral phosphate exit.

Regulation of phosphate transport at the proximal tubule apical membrane is precise because this is the only and final site of determination of extracellular phosphate balance by the kidney. Phosphate uptake is affected by incoming signals, such as parathyroid hormone (55), dopamine (56), fibroblast growth factor-23 (57), and Klotho (58), which inhibit phosphate transport and induce phosphaturia. One of the most potent regulators of phosphaturia is dietary phosphate intake itself, which may involve a variety of hormones, including unknown intestinal enterokines (59), and direct sensing by the proximal tubule (60). This is one of the most important, yet least known, areas in phosphate homeostasis. The modulation of proximal phosphate transport is achieved largely by trafficking of the transporters in and out of the apical membrane (52) with the exception of Klotho, which can directly affect phosphate transport activity (58).

Response to Acidosis

The catabolism of acidic and sulfur-containing amino acids results in the net production of acids. As a result, a high-protein diet leads to a mild chronic metabolic acidosis that is usually well compensated. The common clinical condition of metabolic acidosis is characterized by a more significant decrease in plasma pH and bicarbonate concentration. This disturbance in acid-base balance can be caused by genetic or acquired alterations in metabolism, in renal handling of bicarbonate, and in the excretion of acid. In addition, patients with cachexia, trauma, uremia, ESRD, and HIV infection frequently develop acidosis as a secondary complication that adversely affects their outcome. Chronic acidosis also causes impaired growth, bone loss, muscle wasting, nephrocalcinosis, and urolithiasis. In acute situations with massive acid production that overwhelms the renal capacity, the kidney’s response is not relevant; however, in more chronic conditions, renal compensation is crucial.

An essential renal compensatory response to metabolic acidosis is initiated by increased extraction and catabolism of plasma glutamine that occur predominantly in the proximal convoluted tubule. The resulting increases in renal ammoniagenesis and transport into the urine accomplish the excretion of acid, whereas the increased bicarbonate synthesis and transport into the blood partially correct the systemic acidosis. These adaptations occur rapidly after acute onset of acidosis and are subsequently sustained by more gradual changes in gene expression.

During normal acid-base balance, the kidneys extract and metabolize very little of the plasma glutamine. Although approximately 20% of the plasma glutamine is filtered, the measured rat renal arterial-venous difference is <3% of the arterial concentration of glutamine (61), and only 7% of the plasma glutamine is extracted by the human kidneys even after an overnight fast (62). Therefore, renal utilization is significantly less than the fraction of plasma glutamine filtered by the glomeruli. To account for the effective reabsorption of glutamine, either the activity of the mitochondrial glutamine transporter or the glutaminase must be largely inhibited or inactivated in vivo during normal acid-base balance.

Acute onset of metabolic acidosis produces rapid changes in the interorgan metabolism of glutamine (63) that support a rapid and pronounced increase in renal catabolism of glutamine. Within 1–3 hours, the arterial plasma glutamine concentration is increased 2-fold (64) due primarily to an increased release of glutamine from muscle (65). Significant renal extraction of glutamine becomes evident as the arterial plasma concentration is increased. Net extraction by the kidney reaches 35% of the plasma glutamine, a level that exceeds the proportion (20%) filtered by the glomeruli. Thus, the normal direction of the basolateral glutamine exchange transporter, LAT2-4F2hc, is reversed in order for the proximal convoluted tubule cells to extract glutamine from both the glomerular filtrate and the venous blood (Figure 5). In addition, the transport of glutamine into the mitochondria may be acutely activated (66). Additional responses include a prompt acidicification of the urine that results from translocation, as evidenced in OKP cells (67), and by acute activation of NHE3 (68). This process facilitates the rapid removal of cellular ammonium ions (69) and ensures that the bulk of the ammonium ions generated from the amide and amine nitrogens of glutamine are excreted in the urine. Finally, the cellular concentrations of glutamate and α-ketoglutarate are significantly decreased within the rat renal cortex (70). The latter compounds are products and inhibitors of the glutaminase and glutamate dehydrogenase reactions, respectively. The decrease in concentrations of the two regulatory metabolites may result from a pH-induced activation of α-ketoglutarate dehydrogenase (69). Thus, the acute increase in renal ammoniagenesis may result from a rapid activation of key transport processes, an increased availability of glutamine, and a decrease in product inhibition of the enzymes of ammoniagenesis.

During chronic acidosis, many of the acute responses are reversed and the arterial plasma concentration is decreased to a new steady state that is 70% of normal. However, more than one third of the plasma glutamine is still extracted in a single pass through the kidneys. The increased renal catabolism of glutamine in the proximal convoluted tubule is now sustained by increased expression of genes that encode key transporters and enzymes of glutamine metabolism (Figure 5). A comprehensive survey of the adaptative response of known amino acid transporters in mouse kidney demonstrated that only the basolateral SNAT3 (SLC38A3) transporter exhibits a rapid and pronounced increase during onset of acidosis (71). The SNAT3 transporter has a high affinity for glutamine (72). Under physiologic conditions, it catalyzes a reversible Na⁺-dependent uptake of glutamine that is coupled to the efflux of H⁺ ions (73). SNAT3 is normally localized solely to the basolateral membrane of the S3 segment of the proximal tubule (74). This segment expresses high levels of glutamine.
synthetase (75). Thus, the SNAT3 transporter may normally facilitate the pH-dependent release of glutamine. However, during chronic acidosis, increased expression of the SNAT3 transporter occurs primarily in the basolateral membranes of the S1 and S2 segments of proximal tubule, the site of increased glutamine catabolism (76). Given the sustained increase in H⁺ ion concentration within these cells, the increased expression of the SNAT3 transporter may now facilitate the basolateral uptake of glutamine and contribute to its sustained extraction during chronic acidosis. Within 8–24 hours after onset of acidosis, a pronounced increase in phosphoenolpyruvate carboxykinase (77) also occurs only in the proximal convoluted tubule. More gradual increases in levels of mitochondrial glutaminase (78,79) and glutamate dehydrogenase (80) that require 4–7 days also occur solely within the proximal convoluted tubule. In addition, the level of aquaporin-8, a potential mitochondrial ammonia transporter, is increased (81).

Glutamine uptake in mitochondria from normal rats is mediated through two glutamine antiporters, whereas a highly active glutamine uniporter is evident only in mitochondria prepared from acidotic rats (82). Therefore, acidosis leads to increased expression or activation of a unique, but unidentified, mitochondrial glutamine transporter. Acute activation and subsequent increase in expression of NHE3 (83–85) acidifies the fluid in the tubular lumen and contributes to the active transport of ammonium ions as a direct substrate of NHE3 (69). The adaptation in NHE3 likely reflects the increased demand for ammonium ion secretion although this is difficult to prove. The filtered HCO₃⁻ load is certainly decreased in metabolic acidosis, so increased HCO₃⁻ absorptive capacity is not required. As a result, increased renal ammoniagenesis continues to provide an expendable cation that facilitates excretion of strong acids while conserving sodium and potassium ions. In rats (86,87) and humans (35), the a-ketoglutarate generated from renal catabolism of glutamine is primarily converted to glucose. This process requires phosphoenolpyruvate carboxykinase to divert oxaloacetate, derived from intermediates of the tricarboxylic acid cycle, into the pathway of gluconeogenesis. The combined

Figure 5. | Renal proximal tubular catabolism of glutamine. (A) During normal acid-base balance, the glutamine filtered by the glomeruli is nearly quantitatively extracted from the lumen of the proximal convoluted tubule and largely returned to the blood. The transepithelial transport utilizes B’AT1, a Na⁺-dependent neutral amino acid cotransporter in the apical membrane, and LAT2, a neutral amino acid antiporter in the basolateral membrane. To accomplish this movement, either the mitochondrial glutamine transporter or the mitochondrial glutaminase (GA) must be inhibited (red X). The apical Na⁺/H⁺ exchanger functions to slightly acidify the lumen to facilitate the recovery of HCO₃⁻ ions. (B) During chronic acidosis, approximately one third of the plasma glutamine is extracted and catabolized within the early portion of the proximal tubule. B’AT1 continues to mediate the extraction of glutamine from the lumen. Uptake of glutamine through the basolateral membrane occurs by reversal of the neutral amino acid exchanger, LAT2, and through increased expression of SNAT3. Increased renal catabolism of glutamine is facilitated by increased expression (red arrows) of the genes that encode glutaminase (GA), glutamate dehydrogenase (GDH), phosphoenolpyruvate carboxykinase (PEPCK), the mitochondrial aquaporin-8 (AQP8), the apical Na⁺/H⁺ exchanger (NHE3), and the basolateral glutamine transporter (SNAT3). In addition, the activities of the mitochondrial glutamine transporter and the basolateral Na⁺/HCO₃⁻ exchanger are increased (+). Increased expression of NHE3 contributes to the transport of ammonium ions and the acidification of the luminal fluid. The combined increases in renal ammonium ion excretion and gluconeogenesis result in a net synthesis of HCO₃⁻ ions that are transported across the basolateral membrane by the Na⁺/HCO₃⁻ cotransporter (NBC1). CA, carbonic anhydrase; aKG, a-ketoglutarate; Mal, malate; OAA, oxaloacetate; PEP, phosphoenolpyruvate.
pathways of ammoniagenesis and gluconeogenesis result in a net production of 2 NH₃ and 2 HCO₃⁻ ions per glutamine. Activation of NBCe1A (83), the basolateral Na⁺/3HCO₃⁻ cotransporter, facilitates the translocation of reabsorbed and de novo-synthesized HCO₃⁻ ions into the renal venous blood. Thus, the combined adaptations also create a net renal release of HCO₃⁻ ions that partially restores acid-base balance.

The adaptive increase in the NaDC-1 transporter contributes to increased reabsorption and metabolism of citrate within the proximal tubule. This reduces the excretion of a valuable base in the urine. This adaptation occurs at multiple levels. Acidification of luminal pH titrates citrate⁵⁻ to citrate⁻²⁻, which is the preferred substrate, and low pH also directly activates NaDC1 to increase transport independent of [citrate⁻²⁻] (26,88). In addition to transport, increased cellular metabolism also drives citrate reabsorption. After cellular uptake, citrate is metabolized through one of two pathways: a cytoplasmic pathway involving citrate lyase or a mitochondrial pathway involving the citric acid cycle (47). During acidosis, the activities of cytoplasmic citrate lyase and mitochondrial aconitase are also increased (89). Because both pathways generate HCO₃⁻, the increased reabsorption of citrate is equivalent to a decrease in base excretion (90). Enhanced catabolism of citrate also produces substrates that support the increased gluconeogenesis.

Acid-base disturbances are a major regulator of proximal tubule phosphate handling. Metabolic and respiratory acidosis increase phosphate excretion and metabolic and respiratory alkalosis decrease phosphate excretion. The mechanism of phosphaturia in acidosis is complex and mediated by many factors, including increased release of phosphate from bone (91), titration of luminal phosphate to monovalent form, direct gating of NaPi-IIa and NaPi-IIc by pH (54), decrease in apical membrane protein (92), and transcripts (93) of the transporters, although disparate results on protein levels have been described (Figure 3B) (94). The acid-induced phosphaturia serves to increase urinary H⁺ buffer but also accommodates the phosphate released from bone associated with acid loading.

The gradual increases in glutaminase (95–97) and glutamate dehydrogenase (98) result from selective degradation of their mRNAs. By contrast, the rapid increase in phosphoenolpyruvate carboxykinase results from enhanced transcription of the PCK-1 gene (99), whereas mRNA stabilization contributes to the sustained increase (100,101). The presence of a pH-response element (pH-RE) that regulates the turnover of the glutaminase mRNA was initially demonstrated by stable expression of a chimeric β-globin reporter mRNA, which includes a 956-bp segment of the 3'-untranslated region of glutaminase mRNA (95). RNA gel shift analyses of various deletion constructs mapped the pH-RE to two 8-nt AU-sequences within the 3'-untranslated region. Mutation of the pH-RE within the β-globin reporter mRNA blocked the pH-responsive stabilization. In addition, insertion of only a 29-bp segment containing the pH-RE was sufficient to produce both rapid degradation and pH-responsive stabilization (102). Therefore, the identified pH-RE contributes to the rapid turnover of the glutaminase mRNA and is both necessary and sufficient to mediate its pH-responsive stabilization.

Proteomic analysis of isolated rat renal proximal convoluted tubules identified an additional 60 proteins that are increased during acute and chronic acidosis (103–105). More than 50% of the mRNAs that encode the induced proteins contain an AU-sequence that has >85% identity with the pH-response element found in the glutaminase mRNA. This finding strongly suggests that mRNA stabilization is a primary mechanism by which protein expression is increased in response to onset of metabolic acidosis.

The maintenance of the composition and content of the extracellular fluid volume is critical to health, as evidenced by the multiple organ failure seen in kidney disease. The mammalian proximal tubule uses an array of polarized membrane transporters to accomplish vectorial solute transport. By lowering the luminal content of many abundant solutes to a level that is within the lower reabsorptive capacity of the distal nephron segments, it enables the absorption of these solutes to be fine-tuned downstream. Without the large proximal reabsorption capacity, it would be impossible for the kidney to maintain the high GFR that is necessary to sustain the high metabolic rates characteristic of terrestrial vertebrates. For many solutes with lower plasma concentrations and, hence, lower filtered load, proximal reabsorption is the final arbitrator of urinary excretion. The kidney has enormous capacity to cope with a wide range of physiologic challenges. Whereas volatile acids can be excreted in a gaseous phase in the lung, the kidney is the only organ where nonvolatile acids can be excreted and where discomposed body buffers, such as bicarbonate, can be regenerated. Metabolic acid loading and metabolic acidosis have undesirable acute and chronic consequences and they trigger a coordinated multiorgan network of adaptive responses that partially rectify the disturbance. Although intuitive in principle, the actual mechanisms are actually extremely complex and the proximal tubule is in the center stage spotlight. With a built-in cast of players inherent to this epithelium, the proximal tubule elicits a complex set of mechanisms that includes intrinsic pH sensing and adaptations in membrane transporters and metabolic enzymes to take a neutral molecule, partition it into an acid and a base, and transfer the acid into urine and the base into the blood. Although the short account in this article summarizes significant advances made over decades, the understanding of this system is just beginning. With the current upsurge of powerful investigative methods, the delineation of mechanisms of adaptation to acidosis will emerge with greater rapidity and clarity.

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