Unraveling the Molecular Pathogenesis of Isolated Proximal Renal Tubular Acidosis

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Abstract. Proximal renal tubular acidosis (pRTA) results from an impairment of bicarbonate (HCO₃⁻) reabsorption in the renal proximal tubules and is characterized by a decreased renal HCO₃⁻ threshold. Proximal RTA most commonly occurs in association with multiple defects of proximal tubular transport (renal Fanconi syndrome). Although much more rare, pRTA may occur without other functional defects in proximal tubules (isolated pRTA). The presenting clinical symptom of isolated pRTA is usually growth retardation in infancy or early childhood. Three categories of isolated pRTA have been identified: (1) autosomal dominant pRTA; (2) autosomal recessive pRTA with ocular abnormalities; and (3) sporadic isolated pRTA. Autosomal dominant and autosomal recessive pRTA are usually permanent; life-long alkali therapy is needed. In contrast, sporadic isolated pRTA is transient; alkali therapy can be discontinued after several years without reappearance of symptoms. Recent genetic studies have begun to elucidate the molecular pathogenesis of inherited isolated pRTA. Studies in knockout mice have identified a candidate gene for autosomal dominant pRTA, SLC9A3, a gene encoding one of the five plasma membrane Na⁺/H⁺ exchangers (NHE3). Patients with autosomal recessive pRTA and ocular abnormalities have recently been found to have mutations in the kidney type Na⁺/HCO₃⁻ cotransporter gene (SLC4A4). Identification of these gene mutations provides new insights into the molecular pathogenesis of pRTA.

Renal tubular acidosis (RTA) is a clinical syndrome characterized by hyperchloremic metabolic acidosis secondary to abnormalities of renal acidification (1). The defect may be a consequence of diminished bicarbonate (HCO₃⁻) reabsorption in the renal proximal tubules, reduced excretion of hydrogen ion (H⁺) in the renal distal tubules, or both (2,3). Classification of RTA has been based exclusively on clinical and functional studies. On the basis of these criteria, RTA can be classified into four main subtypes: distal RTA, proximal RTA, combined proximal and distal RTA, and hyperkalemic RTA. Distal RTA (dRTA or type 1 RTA) is caused by the defect of H⁺ secretion in the distal tubules and is characterized by the inability to maximally acidify the urine below pH 5.5 during systemic acidemia (3). Proximal RTA (pRTA or type 2 RTA) is caused by an impairment of bicarbonate reabsorption in the proximal tubules and is characterized by a decreased renal HCO₃⁻ threshold (4). Distal acidification mechanisms are intact in pRTA. Manifestations of combined proximal and distal RTA (type 3 RTA) are a striking reduction in tubular reclamation of filtered HCO₃⁻ and an inability to maximally acidify the urine in the face of severe acidemia (5). Hyperkalemic RTA (type 4 RTA) is characterized by a normal ability to acidify the urine after an acid load, but net acid excretion remains subnormal due to very low rates of NH₄⁺ excretion (5). Hyperkalemic RTA may occur as a result of aldosterone deficiency or tubular insensitivity to aldosterone. Hyperkalemic RTA with decreased urinary potassium excretion may also occur despite normal aldosterone production in children with obstructive uropathy (6).

In childhood, most forms of isolated dRTA and pRTA are hereditary. In contrast, RTA in adulthood is predominantly caused by autoimmune diseases. For example, renal acidification defects can be demonstrated in as many as 50% of patients with Sjögren syndrome and hyperglobulinemic purpura (7).

Renal Control of Acid-Base Balance

Hydrogen ion balance is tightly regulated as the activity of almost all enzyme systems is influenced by H⁺ concentration. Immediate defense against a change in pH is accomplished by combination of H⁺ with chemical buffers. However, the buffers do not remove H⁺ from the body; rather, they bind it until secondary excretion can occur as a result of respiration or renal excretion. HCO₃⁻ is the major buffer in the extracellular fluid. HCO₃⁻ is filtered by the glomerulus, and H⁺ is secreted into the tubular lumen. Net endogenous noncarbonic acid production is approximately 1 mEq/kg per d in adults and 1 to 3 mEq/kg per d in infants and children (1,5). Net acid excretion is equal to ammonium excretion plus titratable acid minus excreted HCO₃⁻.
Mechanisms of HCO$_3^-$ Reabsorption

Our current understanding of the mechanisms that regulate HCO$_3^-$ reabsorption in the proximal tubule is summarized in Figure 1 (1,5). HCO$_3^-$ is freely filtered from the glomerulus into the tubular lumen. More than 85% of the filtered HCO$_3^-$ is subsequently reabsorbed by proximal tubules. This is accomplished by secretion of H$^+$ into the proximal tubular lumina by secondary active transport. Filtered HCO$_3^-$ combines with H$^+$ to form carbonic acid (H$_2$CO$_3$), which, in the presence of carbonic anhydrase IV (CAIV) in the brush border membrane, quickly dissociates to form CO$_2$ and H$_2$O (1). CO$_2$ in the glomerular ultrafiltrate diffuses into the proximal tubular cells, where hydroxylation occurs to form HCO$_3^-$ in the presence of soluble cytoplasmic carbonic anhydrase II (CAII) (2). Both CAII and CAIV are markedly stimulated during chronic metabolic acidosis. Hydrogen ion secretion in the proximal tubules is mediated by H$^+$-ATPase and electroneutral Na$^+$/H$^+$ exchanger isoform 3 (NHE3) in the luminal membrane. This exchange is driven by a sodium concentration gradient that is maintained by the basolateral Na$^+$/K$^+$-ATPase activity. Three intracellular HCO$_3^-$ and one Na$^+$ are cotransported into blood by the kidney type Na$^+$/HCO$_3^-$ cotransporter (kNBC1) present in proximal tubular basolateral membranes. Luminal bicarbonate concentration and pH, luminal flow rate, peritubular PCO$_2$, and angiotensin II are important determinants of the rate of HCO$_3^-$ reabsorption.

Molecular Biology of Proximal Tubular HCO$_3^-$ Transporters and Regulatory Enzymes

Na$^+/H^+$ Exchanger Isoform 3 (NHE3)

NHE are vital transmembrane transporters that participate in the regulation of intracellular pH and volume as well as trans-epithelial ion transport (8). NHE catalyze the electroneutral one-to-one exchange of Na$^+$ and H$^+$. Seven isoforms (NHE1–7) of this gene family have been cloned in mammals. These isoforms differ in tissue localization, sensitivity to inhibitors, and mode of transcriptional and posttranscriptional regulation. These differences result in functional diversity among the isoforms. All NHE isoforms share a similar topology: an N-terminus of 12 transmembrane (TM) α-helices that collectively function in ion exchange and a C-terminal cytoplasmic regulatory domain that modulates transport activity by the TM domain. Extracellular signals, mediated by diverse classes of cell-surface receptors, regulate NHE1 activity through distinct signaling networks that converge to directly modify the C-terminal regulatory domain. Modifications in the C-terminus, including phosphorylation and the binding of regulatory proteins, control transport activity by altering the affinity of the TM domain for intracellular H$^+$ (9). NHE3 is the principal isoform involved in renal tubular transport and is specifically localized to the apical membrane of proximal tubular epithelial cells and cells of the thick ascending limb of Henle’s loop (TALH) cells (10). The expression of NHE1 is ubiquitous. NHE2 is predominantly expressed in kidney, intestine, stomach, and adrenal gland. NHE4 is mainly expressed in stomach. NHE5 is specifically expressed in brain (11). The human NHE3 gene (SLC9A3) maps to chromosome 5p15.3 (12).

Kidney Type Na$^+$/HCO$_3^-$ Cotransporter (kNBC1)

Na$^+$/HCO$_3^-$ cotransporters (NBC) are a family of integral membrane proteins that mediate electroneutral and electrogenic sodium bicarbonate cotransport (13). Members of the NBC protein family mediate the transepithelial transport of Na$^+$ and HCO$_3^-$ in several tissues and contribute to intracellular pH regulation. The electrogenic kidney type Na$^+$/HCO$_3^-$ cotransporter (kNBC1) is derived from the kNBC1 gene (SLC4A4). kNBC1 is expressed in the renal proximal tubules, where it mediates the majority of basolateral bicarbonate reabsorption. kNBC1 is located primarily in the basolateral membrane of proximal tubules, with only traces found in medullary TALH. The kNBC1 transcript is also expressed in corneal endothelial cells and duodenal epithelial cells. A homologous protein, encoded by a mRNA splice variant of the same gene (SLC4A4), the pancreatic-type Na$^+$/HCO$_3^-$ cotransporter (pNBC1), is highly expressed in pancreas, with lower levels of expression in brain, spinal cord, kidney, colon, thyroid, and prostate. At the protein level, the NBC family is 30 to 35% identical to the anion exchanger (AE) family. Deduced amino acid sequences indicate that NBC and AE families share many regions of identity, both in the putative membrane spans and in the putative cytoplasmic regions. kNBC1 expression is upregulated in metabolic acidosis, potassium depletion, and adrenocorticosteroid excess (14).

kNBC1 mRNA encodes a protein of 1035-amino acid residues (15). The kNBC1 protein contains ten transmembrane domains and two intracytoplasmic termini (Figure 2). In contrast, the pNBC1 mRNA encodes a 1079–amino acid residue. pNBC1 mRNA is almost identical to the kNBC1 mRNA except that pNBC1 mRNA has a unique 5’ open reading frame that encodes 85 amino acids that replace the first 41 amino acids of the kNBC1 mRNA transcribed from an alternative promoter in intron 3 of the pNBC1 gene (16). The human SLC4A4 gene is located on chromosome 4p21 (17).

Figure 1. Model of luminal acidification by proximal tubular cells. CA, carbonic anhydrase; ATPase, adenosine triphosphatase. Shaded boxes represent the proteins that are discussed in detail in the article.
Carbonic Anhydrases II and IV

Carbonic anhydrase (CA) are zinc-containing metalloprotein enzymes that catalyze the interconversion of CO$_2$ to HCO$_3^-$ (20). The mechanism of the catalyzed reaction has involved two separate steps. The first step is the reaction of CO$_2$ with an OH$^-$/H$^+$ bound in the fourth ligand position on the zinc. This leads to formation of HCO$_3^-$ (21). The HCO$_3^-$ then diffuses away while the fourth ligand position on the zinc becomes occupied by a water molecule. The second step involves the transfer of a proton from the ligand water molecule, most likely to one of the neighboring histidine residues, which causes the water ligand to reform OH$^-$. A buffer is then required in solution to carry the proton from the histidine residue (22). The net result of this reaction is as follows:

\[ \text{CO}_2 + \text{OH}^- = \text{HCO}_3^- \]

The conversion of HCO$_3^-$ to CO$_2$ + OH$^-$ involves the above mechanism in reverse (21). The CA gene family currently comprises more than ten enzymatically active members. They are major players in many physiologic processes, including renal and male reproductive tract acidification, bone resorption, and formation of gastric acid. CA isozymes have different kinetic properties, tissue-specific distribution patterns, and variable intracellular compartmentalization. CAI, II, III, and VII are cytoplasmic, CAV is mitochondrial, and CAVI is found extracellularly in salivary secretions (19). CAIV, IX, XII, and XIV are membrane proteins; CAIV is a glycosylphosphatidylinositol-anchored protein and CAIX, XII, and XIV are transmembrane proteins. More than 95% of the total body CA activity is located in the cytosol and is attributable to CAII in the kidney. CAII is highly expressed in intercalated cells of the collecting duct, and it is expressed at lower levels in proximal tubules, loop of Henle, and collecting duct principal cells (20). CAIV is present on the apical brush-border membrane and on the basolateral membrane of proximal tubule cells. CAIV has been thought to be represented approximately 5% of total kidney CA activity (21). Recent evidence suggests that other isoforms of CA are also involved (22). The human CAII gene (CA2) maps to chromosome 8q22; the human CAIV gene (CA4) maps to chromosome 17q23 (23).

Proximal HCO$_3^-$ reabsorption is influenced by luminal HCO$_3^-$ concentration, urinary flow rate, extracellular fluid volume, peritubular HCO$_3^-$ concentration, and PCO$_2$, Cl$^-$, K$^+$, Ca$_{2+}$, phosphate, parathyroid hormone, glucocorticoids, adrenergic tone, and angiotensin II (24). Acid loading induces CAII and CAIV mRNA expression in proximal tubules (25). Autosomal recessive mutations of the CAII gene have been described (26,27). CAII is present in the cytosol of both proximal and distal tubules; these mutations therefore lead to a syndrome consisting of pRTA and/or dRTA. Additional manifestations include osteopetrosis, cerebral calcifications, and mental retardation (26,27).

Clinical Features of Patients with Isolated pRTA

A defect in proximal tubules only involving the reabsorption of HCO$_3^-$ (isolated pRTA) is rare. Patients usually come to medical attention due to growth retardation in infancy or early childhood. Isolated pRTA can be divided into three sub-categories: (1) autosomal dominant pRTA; (2) autosomal recessive pRTA with ocular abnormalities; and (3) sporadic isolated pRTA. Autosomal dominant and autosomal recessive pRTA are usually permanent; life-long alkali therapy is needed. In
contrast, sporadic isolated pRTA is transient; alkali therapy can be discontinued after several years without reappearance of symptoms.

**Autosomal Dominant pRTA**

This type of pRTA has only been reported in a single Costa Rican family. Nine affected members from several generations presented with hyperchloremic metabolic acidosis with a plasma HCO$_3^-$ concentration in the range of 11.3 to 17.9 mM/L and urine pH <5.2 (28). Plasma creatinine levels were normal. Net fixed acid production was normal and appropriate for their body weights, averaging 0.9 and 10.2 mEq/kg per d. Acid balance was near zero, at 1.9 ± 2.3 and −2.2 ± 2.2 mEq/d. Urine calcium excretion was normal (29). Associated clinical findings were limited growth retardation and reduced bone densities. Pedigree analysis suggested an autosomal dominant inheritance pattern.

**Autosomal Recessive pRTA with Ocular Abnormalities**

Autosomal recessive proximal RTA is a rare disorder associated with severe short stature (height, −4.4 to −5.0 SD scores) and ocular abnormalities such as glaucoma, cataracts, and band keratopathy (Figure 3). Additional features may include enamel defects of the permanent teeth, psychomotor retardation, and impaired intellectual capacity (30,31,32). Head computed tomography (CT) scan may demonstrate calcification of the basal ganglia (Figure 4) (32). Biochemical hypothyroidism and hyperamylasemia have also been noted in a few patients. This disorder was initially considered to be inherited by an X-linked mechanism because the first reported patients were brothers (33). However, subsequent studies of patients identified by ourselves (32) and Donckerwolcke et al. (30) suggested an autosomal recessive pattern of inheritance. The patients were female, and consanguinity was known for one family (32).

Metabolic abnormalities of severe metabolic acidosis (pH, 7.07 to 7.20; plasma HCO$_3^-$, 5.0 to 10.4 mM/L) and hypokalemia (K, 2.6 to 3.3 mEq/L) were linked to an abnormally low renal threshold for HCO$_3^-$ reabsorption. We have recently
reported an additional patient for whom long-term correction of acidaemia with alkali therapy (10 yr) improved height growth velocity, even though the acidaemia was not fully corrected (34).

Ocular manifestations have been reported in all patients and may progress with age. One of our patients became legally blind when she was 22 yr old (35). An unusual ocular phenotype, one of our patients developed glaucoma without cataracts or keratopathy at 24 yr of age (36).

**Sporadic Isolated pRTA**

A non-familial transient type of isolated pRTA has been reported during infancy (3,4). Patients had defective renal and intestinal CO$_3^-$ reabsorption without an identifiable cause in the absence of other abnormalities. The patients had growth retardation and recurrent vomiting during early infancy. Alkali therapy normalized their growth and was discontinued after several years without reappearance of any symptoms.

**Molecular Basis of Isolated pRTA**

**Autosomal Dominant pRTA**

Genetic studies in families with autosomal dominant pRTA have not yet been reported. However, the gene encoding the NHE3 (SLC9A3) is considered a strong candidate (37). SLC9A3 knockout mice have been generated and shown to lack NHE3 activity. These mice have combined renal and intestinal defects of HCO$_3^-$ reabsorption (38). Microperfusion and micropuncture studies documented a significant decrease of HCO$_3^-$ reabsorption in the proximal tubule (39,40). However, the mice only exhibit a mild degree of metabolic acidosis, due to a compensatory increase of HCO$_3^-$ reabsorption in more distal nephron segments.

**Autosomal Recessive pRTA with Ocular Abnormalities**

Patients with permanent isolated pRTA associated with ocular abnormalities have mutations in the gene encoding kNBC1 (35,36). Three unrelated female patients were found to have three different homozygous mutations: homozygous C to A transition at nucleotide 1043, resulting in the substitution of amino acid from arginine (R) to serine (S) at codon 298 (R298S mutation); homozygous nonsense G to A transition at nucleotide 1678, resulting in the amino acid substitution from arginine (R) to histidine (H) at codon 510 (R510H mutation); and a homozygous nonsense C to T transition at nucleotide 234, resulting in the formation of a stop codon at codon 29 (Q29X mutation). The R298S and R510H missense mutations decreased the functional activity of kNBC1 to 55 to 57% of the wild-type activity when assayed in a eukaryotic expression system (35). Clinically, in addition to pRTA with hypokalaemia, both of these patients were mentally retarded with severe short stature. Ocular abnormalities included glaucoma, cataracts, and band keratopathy in both patients. The patient with the homozygous Q29X nonsense mutations manifested only bilateral glaucoma; she did not have cataracts or band keratopathy.

More recently, another homozygous missense mutation has been detected in a patient with pRTA, short stature, enamel defects of the teeth, bilateral glaucoma, and cataracts (41). The mutation due to a C to T transition at nucleotide 1280 results in the amino acid substitution of from serine (S) to leucine (L) at codon 427 (S427L). Mechanistic studies of kNBC1 proteins are under way in Xenopus oocytes (42,43). The R298S and R510H missense mutations are expected to affect the transcripts for the genes encoding both the kNBC1 and the pNBC1 proteins (Figure 5). Although pancreatic fluid HCO$_3^-$ and pH were not measured in any of these patients, elevated serum amylase levels were observed in the patient with R298S mutation raising the possibility of a pancreatic phenotype. In addition to decreased kNBC1 activity, both mutant proteins were shown to be associated with decreased pNBC1 activity to approximately half of the wild-type level when assayed in a eukaryotic expression system (unpublished data). On the other hand, the Q29X nonsense mutation, which occurred in the unique 5'-end of kNBC1 cDNA, is predicted to yield a truncated kNBC1 protein that lacks 1007 N-terminal amino acids. The predicted effect is complete loss of function of the kNBC1 protein, whereas the pNBC1 should be unaffected (Figure 5).

Basolateral transport of HCO$_3^-$ in the proximal tubules is mediated predominantly by kNBC1; inactivating mutations in the kNBC1 transcript can therefore account for the renal phenotype in this disorder. The ocular abnormalities can also be ascribed to inactivating mutations in the NBC gene. The ocular abnormalities can also be ascribed to inactivating mutations in the NBC gene. The presence of sodium bicarbonate cotransport activity has been detected in several ocular tissues (44–46),
although the molecular basis of this transport has not been established. We demonstrated that human corneal endothelial cells express both kNBC1 and pNBC1 mRNA (47). Using isoform-specific antibodies, we further established that both kNBC1 and pNBC1 protein are expressed in several ocular tissues such as corneal endothelium, trabecular meshwork, and lens epithelium (48). In addition, adenovirus-mediated delivery of a specific hammerhead ribozyme against NBC to human lens epithelial cells largely suppressed NBC protein expression and sodium bicarbonate cotransport activity (48). The expression of NBC isoforms in multiple ocular tissues has also been confirmed by others (49).

On the basis of these observations, we have proposed the following hypothesis for the pathogenesis of the ocular abnormalities observed in patients with autosomal recessive pRTA. In the corneal endothelium and lens epithelium, NBC is involved in net fluid transport. In particular, the corneal endothelium transports Na\(^+\), HCO\(_3^-\), and fluid out of the corneal stroma into the aqueous humor (50). The inactivation of NBC1 could increase the HCO\(_3^-\) concentration in the corneal stroma and facilitate Ca\(^{2+}\) deposition in the Bowman membrane, leading to band keratopathy. The lens epithelium also actively transports fluid from the anterior to the posterior side of the lens (51). The inactivation of NBC could disrupt homeostasis of the lens by affecting this active transport, resulting in cataract formation. On the other hand, the pathogenesis of glaucoma is less obvious. However, the trabecular meshwork is the main site for aqueous humor outflow (52); it is therefore tempting to speculate that inactivation of NBC could alter the contractile properties of the trabecular meshwork cells leading to increased resistance to aqueous humor outflow. The functional significance of NBC1 in the ciliary epithelium remains to be established. At present, the reason why the patient with the homozygous Q29X nonsense mutation developed glaucoma without band keratopathy or cataracts is unknown.

It has recently been confirmed that kNBC1 and pNBC1 are also expressed in the brain (53). NBC expression is widespread throughout the cerebellum, cortex, olfactory bulb, and subcortical structures. In addition, the temporal expression profile suggests that NBC activity may be critical during the later stages of brain development (54). A malfunction of NBC in the brain could be related to the basal ganglia calcification and mental retardation accompanying pRTA, although the mechanisms remain to be determined.

Sporadic Isolated pRTA

The transient nature of the renal acidification defect in patients with sporadic isolated variant of isolated pRTA has not yet been investigated. It is tempting to speculate that it is due to immature NHE3, H\(^+\)-ATPase, kNBC1, and/or CA activity.

References
