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Review

Renal tubular acidosis — underrated problem?

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Renal tubular acidosis (RTA) is a hyperchloremic metabolic acidosis characterized by a normal anion gap and normal (or near normal) glomerular filtration rate in the absence of diarrhoea. Inherited isolated forms of renal tubular acidosis are not common. However, they can also be a part of a more generalized tubule defect, like in Fanconi syndrome. In recent years more and more gene mutations have been found which are associated with RTA (mutations in the gene *SLC4A4,* encoding a Na^+-HCO_3^- cotransporter (NBC-1); in the gene *SLC4A1*, encoding Cl^-/HCO3$^-$ exchanger (AE1); in the gene *ATP6B1*, encoding B1 subunit of H^+-ATPase; in the gene *CA2* encoding carbonic anhydrase II; and others) and allow better understanding of underlying processes of bicarbonate and H^+ transport. Isolated renal tubular acidosis can be frequently acquired due to use of certain drug groups, autoimmune disease or kidney transplantation. As the prevalence of acquired forms of RTA is common, new therapeutic options for the currently used supplementation of oral alkali, are awaited.

Key words: renal tubular acidosis, molecular pathophysiology, gene mutations, kidney transplantation

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INTRODUCTION

In an adult human, 50–100 mmol of H^+ are generated every day. Main sources of acids include the metabolism of aminoacids such as cystine, cysteine, methionine, lysine, arginine, the metabolism of phospholipids, glucose and fatty acids. The role of kidney in maintaining normal acid-base balance may be divided into two main processes: 1) reabsorption of filtered HCO_3^-, which takes place fundamentally in the proximal tubule, and 2) excretion of net protons (H^+) as titratable acid and ammonium, which occurs in the distal nephron (Fry & Karet, 2007; Ring *et al.*, 2005).

Reabsorption of bicarbonate can increase in the presence of hypovolaemia (due to the action of angiotensin II), hypokalemia, acidosis, hypercapnia, hyperaldosteronism. Conversely, hypervolaemia, hyperkalemia, alkalosis, hypoaldosteronism, and also increased PTH level, decrease the reabsorption of bicarbonate.

Interestingly, the food contents can play a role in bicarbonate amount in the body. It is assumed that sources of potassium in food (predominantly plants) are, in general, potential sources of bicarbonate (Demigne, 2004).

Proximal tubule HCO_3^- reabsorption

Eighty to ninety percent of freely filtered HCO_3^- is reabsorbed in the proximal tubule. In the lumen, filtered HCO3$^-$ reacts with H^+ to form carbonic acid, which, in the presence of membrane-bound carbonic anhydrase (CAIV) promptly dissociates into CO_2 and water. CO_2 freely diffuses into the proximal tubular cells where it is combined with water to produce intracellular H^+ and HCO_3^- in the presence of cytoplasmic carbonic anhydrase II (CAII). HCO_3^- is cotransported with Na^+ into blood *via* NBC-1. The intracellular H^+ produced by CAII is secreted into the lumen mainly *via* the Na^+-H^+ exchanger (NHE-3) located on the luminal membrane. Proximal tubular cells are also able to generate bicarbonate and ammonia through the deamination of glutamine to glutamate (Laing *et al.*, 2005).

The mechanisms of proximal tubule bicarbonate reabsorption are presented in Fig. 1 (from Pereira *et al.*, 2009).

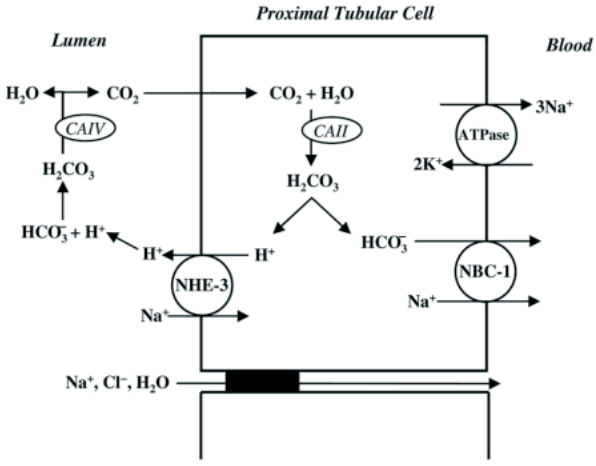

Figure 1. Schematic presentation of bicarbonate (HCO_3^-) proximal reabsorption
CA II, cytoplasmic carbonic anhydrase; CA IV, membrane-bound carbonic anhydrase; NHE-3, Na^+-H^+-exchanger; NBC-1, Na^+-HCO_3^- cotransporter. Adapted from Pereira *et al.*, 2009.

Distal tubule and collecting duct acid secretion

In the distal nephron three processes may contribute to urinary acidification: 1) reclamation of 10–20% of filtered HCO_3^- that is not reabsorbed by proximal tubules, 2) titration of divalent basic phosphate (HPO_4^{2-}) which is converted to the monovalent acid form ($H_2PO_4^-$)

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Abbreviations: ACEI, angiotensin-converting enzyme inhibitor; AIDS, aquired immune deficiency syndrome; ARB, angiotensin receptor blocker; CNI, calcineurin inhibitor; PHA1, primary pseudohypoaldosteronism type 1; PHA2, pseudohypoaldosteronism type 2; RTA, renal tubular acidosis; SLE, systemic lupus erythematosus; UAG, urine anion gap.

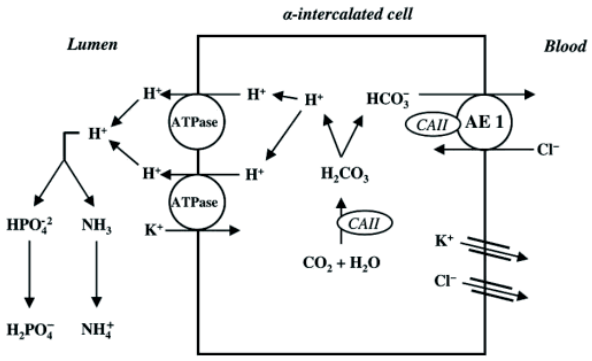

Figure 2. Schematic presentation of α-intercalated cell and H^+ secretion in cortical collecting tubule
AE1, anion exchanger; CA II, cytoplasmic carbonic anhydrase. Adapted from Pereira *et al.*, 2009

or titrable acid, 3) accumulation of NH_3 in the lumen, which buffers H^+ to form ammonium (NH_4^+). In the collecting tubule the α-intercalated cells are responsible for H^+ secretion. These cells secrete H^+ into the lumen not only *via* H^+-ATP-ase, but also by an exchanger (H^+/K^+-ATP-ase). They also transport HCO_3^- *via* Cl^-/HCO_3^- exchanger AE1, which is homologous with the red cell anion exchanger (Laing *et al.*, 2005).

The acidification process in α-intercalated cells of the distal nephron is shown in Fig. 2 (from Pereira *et al.*, 2009).

DIAGNOSTIC APPROACH

Clinical and functional studies allow classification of RTA into four types, numbered in the order of discovery: proximal RTA (type 2), distal RTA (type 1), hyperkalemic distal RTA (type 4) and combined proximal and distal RTA (type 3).

All types of RTA can be characterized by the metabolic acidosis with the presence of a normal anion gap and subsequently, hyperchloremia. The patient should not present gastrointestinal HCO_3^- losses and not be taking drugs such as acetazolamide. The next step requires the evaluation of urine anion gap (UAG). UAG (Na^+ + K^+-Cl^-) provides estimation of urinary ammonium secretion. In physiological conditions, UAG is positive due to the presence of small amounts of unmeasured anions (sulfates, phosphates). In metabolic acidosis any increase in $NH4^+$ excretion is accompanied by a parallel increase in Cl^-, thus UAG is more negative. Apart from patients with proximal RTA, the others present with positive UAG.

In a patient with a negative UAG the definite diagnosis of proximal RTA is established with a high (>15%) urine HCO_3^- excretion at normal plasma HCO_3^- concentration.

In a patient with a positive UAG measuring of plasma K^+ concentration would be the next step in the diagnosis. In case of normal or lower K^+ values, the inability to lower the urine pH below 5.5 after NH_4Cl load or furosemide administration establishes the diagnosis of distal RTA. When the value of plasma K^+ is increased, the finding of urine pH higher than 5.5 after NH_4Cl load will identify patients with hyperkalemic distal RTA caused by a 'voltage-dependent' defect. When the urine pH is lower than 5.5, the diagnosis of hyperkalemic RTA (type 4) is confirmed. The presence of a moderate degree of HCO_3^- wasting (5 to 15% of fractional HCO_3^- excretion at normal plasma HCO_3^-) indicates type 3 RTA (Unwin, 2001).

The diagnostic work-up in a patient with the suspicion of RTA is presented in Fig. 3 (from Soriano, 2002).

GENETIC CAUSES OF RTAS

In recent years remarkable progress has been made in the unraveling of the molecular pathogenesis of hereditary diseases caused by mutations in genes encoding transporters in renal tubule.

Inherited forms of distal RTA

The inherited forms of distal RTA include three variants: autosomal dominant and autosomal recessive with or without deafness (Karet, 2002).

Autosomal dominant distal RTA has been found to be associated with mutations in the *SLC4A1* gene encoding $Cl^-HCO_3^-$ exchanger AE1. AE1, an integral membrane glycoprotein, is predominantly expressed in erythrocytes (eAE1) and in the kidney (kAE1). Because of the expression of AE1 in two different cell types (red blood cells and distal tubular cells), *SLC4A1* mutations can

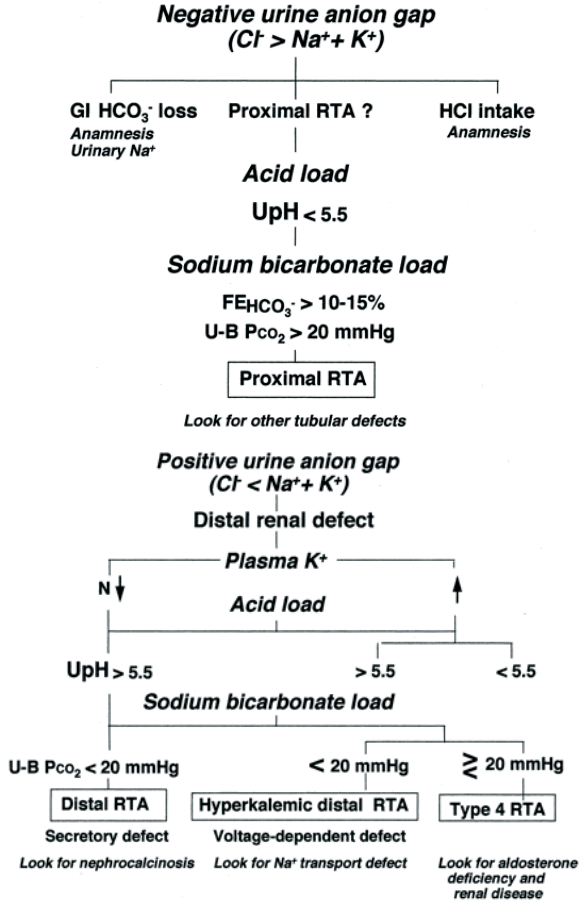

Figure 3. Diagnostic work-up of a patient with hyperchloremic metabolic acidosis with a negative (A) and positive (B) urine anion gap
(A) GI, gastrointestinal; RTA, renal tubular acidosis; UpH, urine pH; FE $_{HCO3-}$, fractional excretion of bicarbonate; U-B PCO_2, urine-to-blood PCO_2 gradient. Adapted from Soriano, 2002. **(B)** RTA, renal tubular acidosis; UpH, urine pH; FE $_{HCO3-}$, fractional excretion of bicarbonate; U-B PCO_2, urine-to-blood PCO_2 gradient. Adapted from Soriano, 2002.

result in two different phenotypes: hereditary spherocytosis (or, in general, erythrocyte abnormalities) and distal RTA (Pereira *et al.*, 2009). The majority of *SLC4A1* mutations cause only erythrocyte abnormalities without renal disorders. A plausible explanation is that the remaining function of the exchanger in heterozygotes may be insufficient to preserve the integrity and function of erythrocytes but sufficient to maintain normal distal H^+ secretion. On the other hand, distal-RTA *SLC4A1* mutations are rarely accompanied by erythrocyte abnormalities. The answer to this phenomenon is probably in the segregation of the mutations; in spherocytosis the mutations are distributed throughout AE1 cytoplasmic and transmembrane domains, whereas distal-RTA mutations are restricted to the AE1's transmembrane domain (Alper, 2002).

Autosomal recessive distal RTA with deafness is related to mutations in the proton pump. The gene involved (*ATP6V1B1*) encodes the B1 subunit of H^+-ATPase in α-intercalated cells (Borthwick *et al.*, 2002). Mutations lead to the disruption of the structure or abrogation of the production of normal B1 subunit protein. H^+-ATPase in human cochlea is necessary to maintain normal endolymph pH. Clinically, there is large variability of deafness severeness with different progression.

Autosomal recessive distal RTA with preserved hearing is a consequence of defective gene *ATP6V0A4* which encodes a kidney-specific a4 isoform subunit of H^+-ATPase. It seems that the a4 subunit is essential for proton pump function in the kidney (Smith *et al.,* 2000).

Inherited forms of proximal RTA

The proximal RTA resulting from Fanconi syndrome is frequently part of a systemic syndrome. The inheritance pattern is usually autosomal recessive and the diseases are: cystinosis, tyrosinaemia, galactosaemia, Fanconi-Bickel syndrome and others.

Primary isolated proximal RTA is a rare disorder (Gross *et al.*, 2008). It can be divided into three categories: 1) autosomal recessive with ocular abnormalities, 2) autosomal dominant 3) sporadic (Igarashi *et al.*, 2002).

Autosomal recessive proximal RTA is a rare disorder with severe growth retardation and ocular abnormalities such as glaucoma, cataracts and band keratopathy which may progress with age. Intellectual impairment and enamel defects of teeth are common. The disorder is caused by mutations of the gene encoding the sodium bicarbonate transporter NBC1 (*SLC4A4*) (Dinour *et al.*, 2004). In consequence, reduced activity of the transporter and/or defects in intracellular trafficking are present. Ocular tissues have also been shown to express NBC1 which can explain the development of ocular abnormalities. NBC1 is also expressed in the pancreas, and some patients with these mutations demonstrate abnormal pancreatic function.

Another inherited form of proximal RTA is the one resulting from mutations in the gene that encodes CAII. CAII is localized in proximal tubular cells and in α-intercalated cells of the cortical and outer medullary collecting tubules. That is why this type of RTA presents with some proximal and distal components (RTA type 3). Clinically, patients present with osteopetrosis, cerebral calcification and mental retardation.

Autosomal dominant proximal RTA was first described in a Costa Rican family, patients presented with growth retardation and osteomalacia. Recently, Katzir *et al.* (2008) described a second family. However, the gene associated with this clinical presentation has not been identified yet.

Recent evidence suggests that a strong candidate for proximal RTA is the *TASK* gene. TASK2-potassium channel seems to be important in bicarbonate reabsorption in renal proximal tubules. Studies on TASK2 gene knock-out mice showed metabolic acidosis with low bicarbonate levels (Warth *et al.*, 2004).

Inherited forms of type 4 RTA

Type 4 RTA is a heterogenous group of disorders associated with hyperkalemia due to aldosterone deficiency or disorders in aldosterone molecular signaling.

Hyperkalemic RTA of hereditary origin is most frequently observed in children with primary pseudohypoaldosteronism type 1 (PHA1). It can have autosomal dominant or recessive form (Hanukoglu, 1991). The autosomal dominant one is a mild kidney disorder without any other organ involvement and is associated with mutation (loss-of-function type) in the mineralocorticoid receptor gene (*MRL* gene). The autosomal recessive PHA1 is related to sodium transport defects not only in the kidney, but also in other aldosterone-target organs, such as colon, lungs or salivary glands. The symptoms are more pronounced.

Pseudohypoaldosteronism type 2 (PHA2) or Gordon's syndrome, is another inherited cause of type 4 RTA (autosomal dominant pattern). Clinical presentation includes hyperkalemia with hypertension and low or normal levels of plasma aldosterone. The basic abnormality is due to gain-of-function mutations in the gene of two isoforms of WNK serine-threonine kinases, *WNK4* and *WNK1* genes (Wilson *et al.,* 2001). WNK4 is found in the distal nephron and regulates sodium and chloride reuptake and inhibits potassium efflux.

Aquired causes of RTAs

Aquired forms of RTA can develop quite commonly (Soriano, 2002). Main causes of secondary RTA are presented in Table 1.

Recently, topiramate, a drug used in the therapy of epilepsy, has been shown to induce RTA. The mechanism involves the inhibition of carbonic anhydrases II and IV (Mirza *et al.*, 2009).

RTA after kidney transplantation

RTA observed in patients after kidney transplantation is usually asymptomatic and subclinical. It may affect even up to 40% of transplanted subjects (Malik *et al.,* 2011). Many different factors have been suggested to be associated with post-transplant RTA: ischemic damage, acute tubular necrosis, graft rejection, the use of calcineurin inhibitors (cyclosporine, tacrolimus). Therefore, RTA that occurs early following kidney transplantation is predominantly a consequence of acute renal failure and disappears quickly (Heering *et al.*, 1996). On the other hand, RTA found in late post-transplant period is usually a result of chronic graft rejection or calcineurin inhibitors (CNI) nephrotoxicity. Kidney graft interstitial inflammation or obstructive nephropathy may play a role in the development of RTA. It has also been suggested that alloantigens trigger a direct immune-mediated mechanism by means of specific interference with molecules taking part in tubular acid handling (Ambuhl *et al.*, 2007). Patients with secondary hyperparathyroidism before transplantation tend to develop post-transplant RTA as PTH causes bicarbonate to leak from proximal tubules.

The use of corticosteroids and CNIs may lead to a defect of ammonia synthesis in the proximal tubule due

Table 1. Causes of secondary renal tubular acidosis

Distal RTA	Calcium disorders (hyperparathyroidism, vitamin D intoxication, idiopathic hypercalciuria) Dysproteinemic syndromes (hypergammaglobulinemia, cryoglobulinemia, amyloidosis) Autoimmune diseases (systemic lupus erythematosus [SLE], Sjogren's syndrome, primary biliary cirrhosis, rheumatoid arthritis) Renal diseases (obstructive nephropathy, Balkan nephropathy, renal transplant rejection) Drugs and toxins (amphotericin B, lithium, trimethoprim)
Proximal RTA	Fanconi syndrome (cystinosis, galactosemia, fructose intolerance, tyrosinemia, Wilson's disease, multiple myeloma, light chain disease) Related to other diseases (vitamin D deficiency, hyperparathyroidism, chronic hypocapnia, medullary cystic disease, corticoresistant nephritic syndrome, renal transplantation, amyloidosis) Drugs and toxins (acetazolamide, aminoglycoside antibiotics, outdated tetracyclins, lead, cadmium, mercury)
Hyperkalemic type 4 RTA	Hyporeninemic hypoaldosteronism (diabetes, SLE, AIDS nephropathy) Chronic intersititial nephropathies Drug-induced hyperkalemia: Impaired renin-aldosterone elaboration (cyclo-oxygenase inhibitors, ACEI, heparin) Inhibitors of renal K+ secretion (trimethoprim, pentamidine, CNIs) Altered K+ distribution (beta-blockers, digitalis)

to insulin resistance, and in consequence to metabolic acidosis.

There are, however, other mechanisms of RTA development during cyclosporine use in renal transplant recipients. The mechanism of action of CNIs involves the reaction between calcineurin-inhibitory complex and cytosolic receptor, cyclophilin A. Cyclosporin also inhibits the peptidyl prolyl *cis-trans* isomerase (PPIase) activity on cyclophilin A (Watanabe *et al.*, 2005). PPIase of cyclophilins is also present in intercalated cortical collecting duct cells and may be inhibited by cyclosporin. In this indirect way the hensin polymerization necessary in transforming intercalated cells from β-type (bicarbonate secretion) to α-type (acid secretion) is also inhibited. Chronic CNI nephrotoxicity leads to interstitial histological damage of the kidney.

Classic distal RTA has been described as the main form of RTA in subjects after kidney transplantation. However, type 4 may also be seen in subjects with diabetes mellitus using angiotensin-converting enzyme inhibitors (ACEI) or angiotensin receptor blockers (ARBs) (Schwarz *et al.*, 2006).

TREATMENT

The aim of RTA treatment is not only to correct the biochemical abnormalities, but also to improve the growth of children and to prevent the development of nephrocalcinosis and chronic kidney disease. The basis of therapy is to administer continually appropriate amounts of alkali in the form of bicarbonate or citrate (Soriano, 2002).

For distal RTA, the amount of administered alkali should be 1–2 mmol/kg/day. However, even more important is the proper supplementation of potassium, as the correction of acidosis can enhance hypokalemia. The preferred form of potassium administration is potassium citrate because of its alkalizing effect and protection against hypocitraturia.

For proximal RTA, the amount of administered alkali is very large (5–15 mmol/kg/day). Administration of potassium salts is advised.

For hyperkaemic RTA, the treatment depends on the underlying cause. Potassium-retaining drugs should always be avoided. Fludrocortisone therapy may be useful in hyporeninemic hypoaldosteronism, preferably in combination with a loop diuretic.

CONCLUDING REMARKS

There are more and more molecular developments concerning mutations in genes encoding transporters or channel proteins in renal tubules that may result in primary RTA. New clinical entities (e.g., kidney transplantation) broaden the spectrum of acquired forms of RTAs. Further studies improving our knowledge on RTA pathophysiology and providing a basis of targeted therapeutic interventions are awaited.

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