Receptor Protein Tyrosine Phosphatase \( \gamma \), \( \text{CO}_2 \) Sensing in Proximal Tubule and Acid Base Homeostasis

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The proper balance between acids and bases in the circulation is essential for systemic pH homeostasis, which, in turn, plays a critical role in the operation of biologic systems, ranging from cellular enzymes to chemical reactions, and encompassing every possible tissue or organ, including cardiac cells, neurons, myocytes, etc. The kidney plays an essential role in systemic pH homeostasis by completely reabsorbing all of the filtered bicarbonate (\( \text{HCO}_3^- \)), with approximately 85%–90% of this function being accomplished in the proximal tubule. How the proximal tubule senses acute changes in systemic carbon dioxide (\( \text{CO}_2 \)) or \( \text{HCO}_3^- \) and how that affects its \( \text{HCO}_3^- \) reabsorption capabilities are poorly characterized. In this issue of the Journal of the American Society of Nephrology, Zhou et al. demonstrate that deletion of the receptor protein tyrosine phosphatase-\( \gamma \) (RPTP\( \gamma \)) abolishes the effect of \( \text{CO}_2 \) and \( \text{HCO}_3^- \)–alteration on \( \text{HCO}_3^- \)–absorbing ability (\( \text{JHCO}_3^- \)) in the proximal tubule. They also demonstrate that mice lacking RPTP\( \gamma \) are unable to recover from systemic acidosis after exposure to a systemic acid load. These results suggest that RPTP\( \gamma \) is likely a novel extracellular \( \text{CO}_2/\text{HCO}_3^- \) sensor critical for pH homeostasis. The key player in pH homeostasis is ubiquitous carbonic anhydrase, which produces instantaneous equilibrium of carbon dioxide (\( \text{CO}_2 \)) and carbonic acid (\( \text{H}_2\text{CO}_3 \)) in biologic systems, causing rapid dissociation into acid (\( \text{H}^+ \)) and bicarbonate (\( \text{HCO}_3^- \)) according to the following reactions:

\[
\text{CO}_2 + \text{H}_2\text{O} \rightleftharpoons \text{H}_2\text{CO}_3 \rightleftharpoons \text{H}^+ + \text{HCO}_3^-.
\]

Regulation of systemic (blood) pH in mammals is dependent on the balance between the concentration of \( \text{H}_2\text{CO}_3 \) and \( \text{HCO}_3^- \) ions (above reaction) according to the Henderson–Hasselbalch equation: \( \text{pH} = 6.1 + \log ([\text{HCO}_3^-]/[0.03 \times \text{pCO}_2]) \). As noted, the ratio of \( \text{HCO}_3^- \) to \( \text{H}_2\text{CO}_3 \) determines the systemic pH, which can alter in response to changes in \( \text{CO}_2 \) or \( \text{HCO}_3^- \) levels. The body will maintain the systemic pH in a narrow physiologic range by removing \( \text{CO}_2 \) through respiration and reabsorbing the filtered \( \text{HCO}_3^- \) and excreting excess \( \text{H}^+ \) via the kidney.

The kidney filters approximately 4200–4500 meq of \( \text{HCO}_3^- \), but is able to reabsorb all of the filtered \( \text{HCO}_3^- \) under baseline conditions by secreting equal amounts of \( \text{H}^+ \) into the tubular fluid.1 The majority of filtered \( \text{HCO}_3^- \) is reabsorbed in the kidney proximal tubule by \( \text{H}^+ \) secretion across the luminal membrane via the sodium (\( \text{Na}^+ \))/hydrogen (\( \text{H}^+ \)) exchanger 3 (NHE-3; SLC9A3) and \( \text{H}^+ \) ATPase, working in tandem with the basolateral \( \text{Na}^+/(\text{HCO}_3^-) \) cotransporter, SLC4A4 (NBC-1; NBC-e1), which cotransports three \( \text{HCO}_3^- \) ions for each \( \text{Na}^+ \) ion.1–4 A number of mutations in the C- and N-termini of NBC-e1 impair its function, resulting in \( \text{HCO}_3^- \) wasting and proximal renal tubular acidosis in humans.5–7 Genetic deletion of NBC-e1 in mice causes a similar phenotype, with very low plasma \( \text{HCO}_3^- \) and arterial pH. While there are no known loss-of-function mutations for NHE-3, the inactivation of NHE-3 in mouse proximal tubule causes \( \text{HCO}_3^- \) wasting and metabolic acidosis.8,9 Taken together, these studies demonstrate that intact and functional NHE-3 and NBC-e1 are crucial for \( \text{HCO}_3^- \) reabsorption in the proximal tubule and systemic acid–base homeostasis.1–9

In order to prevent dramatic changes in intra- or extracellular pH, cells must be capable of sensing and responding to the levels of \( \text{CO}_2 \), \( \text{HCO}_3^- \), and \( \text{H}^+ \) in their surrounding environment. These sensors can trigger appropriate responses in their vicinity, such as the modulation of signaling molecules/enzymes, alteration in membrane potential, or modification of the phosphorylation state of transporters, with the ultimate goal of altering the activity of acid-base transporters and maintaining the ratio of \( \text{H}_2\text{CO}_3 \) and \( \text{HCO}_3^- \), hence preventing dramatic changes in pH.

Sensing pH

There are several bio-sensing molecules expressed in various tissues, including kidney cells, that sense changes in pH, \( \text{CO}_2 \), or \( \text{HCO}_3^- \). Sensing the systemic pH (or \( \text{H}^+ \) concentration in the extracellular space) is predominantly mediated via G protein-coupled receptors (GPCRs) or \( \text{H}^+ \)-sensitive ion channels. At least three GPCR molecules are activated by acidic pH (increased \( \text{H}^+ \) ion concentration) and may in turn activate certain signaling molecules and transporters.10
These include: (1) ovarian cancer GPCR 1 (OGR1, also known as GRPR68), (2) GPR4 (also known as GRPRC6.1), and (3) T cell death–associated gene (8TDAG8, GPR65). In addition to the H⁺-sensing GPCRs, systemic acidity is also sensed via two H⁺-sensitive ion channels: transient receptor potential channels and H⁺-sensing ion channels.⁷ Although some of these molecules are expressed in the kidney, none seems to be a pH sensor in the proximal tubule.

Interestingly, in addition to the extracellular (systemic) pH sensors (above), kidney proximal tubule cells express Pyk2, which is located in the cytoplasm, activated by intracellular acidosis, and consequently stimulates the apical Na⁺/H⁺ exchanger NHE-3 (SLC9A3) and the basolateral Na⁺:HCO₃⁻ cotransporter 1 NBC1 (SLC4A4), with the net effect of enhancing H⁺ secretion into the lumen and absorbing HCO₃⁻.¹¹ Pyk2 is a member of the focal adhesion kinase family of tyrosine kinases and its activation is followed by the activation of tyrosine kinase c-Src.¹¹,¹²

SENSING CO₂/HCO₃⁻

There are very few CO₂/HCO₃⁻-sensing molecules that are also expressed in the kidney. One notable molecule is the soluble adenylyl cyclase (sAC; also known as soluble adenyl cyclase 10, ADCY10, or Sacy), which is primarily expressed in the cytoplasm but is also found in the organelles of cells. sAC is a potential HCO₃⁻ sensor and is a source of cAMP, which affects several ion-transporting processes along the length of the nephron.⁶ In the kidney, sAC is primarily expressed in cells of the thick ascending loop of Henle, the distal tubule, and the collecting duct.¹⁰,¹² Its localization in the proximal tubule remains controversial. An interaction between sAC and Pyk2 has been proposed, which could indicate a potential role for sAC in regulating NHE-3 and NBC-1 in the proximal tubule. Additional studies are needed to firmly establish the role of sAC as a candidate to integrate changes in systemic pH alterations with ion transporters in the kidney proximal tubule.

In mammals, CO₂ is sensed in chemoreceptors and kidney, respiratory, and several other tissues. The difficulties associated with differentiating between the direct effects of CO₂ and the effects of pH and/or HCO₃⁻ may have complicated the design of experiments to test CO₂ sensing by kidney cells. The use of out-of-equilibrium CO₂/HCO₃⁻ solutions, established in the laboratory of the senior author of the current studies, is one of the few ways in which this issue has been examined.¹³,¹⁴ Using this approach, inhibitors of tyrosine kinases were found to promote H⁺ secretion/HCO₃⁻ reabsorption in response to basolateral (blood) CO₂ in the kidney proximal tubules.¹⁴ These experiments suggest that molecules distinct from sAC or the Pyk2/c-Src pathway are responsible for activating apical H⁺ secretion and HCO₃⁻ reabsorption in response to basolateral CO₂. No CO₂ chemosensor molecule has been unambiguously identified in kidney proximal tubule cells.

In the present studies, Zhou et al. demonstrate the localization of RPTPγ on the blood-facing domain of the basolateral membranes of proximal convoluted tubule cells.¹⁵ In isolated proximal tubules from RPTPγ-knockout (KO) mice, the authors found that the dependence of JHCO₃⁻ on basolateral CO₂ concentration was abolished; however, the effect of basolateral HCO₃⁻ on JHCO₃⁻ remained comparable in wild-type and mutant mice.¹⁵ The authors concluded that RPTPγ appears to be a novel extracellular CO₂/HCO₃⁻ sensor in proximal tubule cells and may be critical for regulating HCO₃⁻ absorption and pH homeostasis.¹⁵

In addition to the impaired ability to sense basolateral CO₂ alteration in proximal tubule cells, RPTPγ-KO mice displayed an inability to recover from ammonium chloride-induced metabolic acidosis.¹⁵ Indeed, blood HCO₃⁻ and pH in RPTPγ-KO mice were significantly lower versus baseline conditions after exposure to ammonium chloride acid load, and in comparison to wild-type mice, which completely recovered from systemic acidosis after 7 days of acid load.¹⁵ Intriguingly, RPTPγ-KO mice displayed significant downregulation of apical Na⁺/H⁺ exchanger NHE-3 expression under baseline conditions.¹⁵ This raises the possibility that RPTPγ can function both as a CO₂ sensor and as a regulator of NHE-3, the main HCO₃⁻-absorbing transporter in the kidney proximal tubule. Taken together, these results may suggest that RPTPγ has two distinct functions. In acute states, it works as a basolateral CO₂ sensor; whereas, in chronic states, RPTPγ may have a more complicated effect on the transcription of several ion transporters on the apical membrane of the proximal tubule, including NHE-3. Whether the CO₂-sensing properties of RPTPγ may ultimately impact NHE-3 expression via the regulation of intracellular signals, such as Pyk2 or other molecules, is intriguing and worthy of experiment. Additional studies examining the effect of inducible deletion of RPTPγ in the proximal tubule on Pyk2/c-Src pathway and/or NHE-3 expression/activity could provide critical information into possible links between the CO₂-sensing mechanism of RPTPγ, metabolic acidosis, and acid-base homeostasis.

DISCLOSURES

None.

REFERENCES


A reduction in the protein content of muscle, observably reflected as loss of muscle mass, is a frequent accompaniment of}

Glucocorticoid-Regulated Kinase: Linking Azotemia and Muscle Wasting in CKD

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A reduction in the protein content of muscle, observably reflected as loss of muscle mass, is a frequent accompaniment of aging and inflammatory and catabolic diseases, including progressive CKD. In patients with CKD, aside from the risk of progression to ESRD, all-cause mortality is an important competing risk and has been related to loss of muscle mass. Progressive resistance training in patients with CKD has been shown to restore muscle hypertrophy (albeit incompletely) and improve health–related quality of life. Oddly, the muscle wasting from advanced CKD seems to involve mechanisms independent of nutrition alone, which was shown in a trial of patients on dialysis given intradialytic parenteral nutrition for an extended period of time. These data would suggest that methods to inhibit ongoing muscle loss in CKD together with augmentation of nutritional intake may improve health–related quality of life and reduce mortality.

Protein turnover is constantly ongoing, with an estimated 4% of total body protein undergoing degradation and synthesis every day in homeostasis. The rate of proteins lost and gained (i.e., the turnover rate) depends on the nature and site of a specific protein’s function within the cell, its distribution in cell and tissue types, and the existing pathologic state. Simply put, a net loss in muscle mass can result from reduced protein synthesis in muscle cells, increased protein breakdown, or the presence of both concurrently. Experimental and clinical data suggest that, although the CKD milieu impairs synthesis of myosin and nonmyosin proteins within myocytes, it may have a greater effect on promoting protein catabolism. Metabolic acidosis, insulin resistance and impaired insulin/IGF signaling, and inflammation, all of which are encountered in CKD, seem to play important and direct roles in the muscle loss of CKD (reviewed comprehensively in ref. 8). Excess proinflammatory cytokine productions (TNF-α, IL-6, IL-1, serum amyloid protein A, and IFN-α) have been associated with muscle protein loss in CKD. Hemodialysis has complex effects on protein synthesis, catabolism, and inflammation, affecting muscle mass in multiple ways. The function of muscle satellite cells, which usually remain quiescent but contribute to myocytes in response to injury, may also be affected by CKD through myogenic regulatory signals, such as Myf-5 and myoblast determination protein-1.

Other than from nonspecific increases in protein catabolism in CKD, specific and targeted ubiquitin–proteosome system (UPS)–mediated degradation of myocyte proteins is well recognized to play a key role in CKD–induced muscle loss. TGF-β as well as myostatin and activin A (of the TGF-β family) and their downstream SMAD2/3 signaling have been shown to suppress AKT phosphorylation and enhance forkhead box O phosphorylation in myocytes. These signals, in turn, stimulate the expression of E3-ubiquitin ligases (Atrogin and MuRF-1) and UPS-mediated proteolysis that is responsible for muscle loss in CKD. Similarily, impaired IGF-1 signals associated with insulin resistance in CKD affect phosphoinositide 3-kinase

See related article, “Role of Receptor Protein Tyrosine Phosphatase γ in Sensing Extracellular CO2 and HCO3−,” on pages 2616–2621.

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