Molecular Mechanisms of Acid-Base Sensing by the Kidney

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ABSTRACT

A major function of the kidney is to collaborate with the respiratory system to maintain systemic acid-base status within limits compatible with normal cell and organ function. It achieves this by regulating the excretion and recovery of bicarbonate (mainly in the proximal tubule) and the secretion of buffered protons (mainly in the distal tubule and collecting duct). How proximal tubular cells and distal professional proton transporting (intercalated) cells sense and respond to changes in pH, bicarbonate, and CO₂ status is a question that has intrigued many generations of renal physiologists. Over the past few years, however, some candidate molecular pH sensors have been identified, including acid/alkali-sensing receptors (GPR4, InsR-RR), kinases (Pyk2, ErbB1/2), pH-sensitive ion channels (ASICs, TASK, ROMK), and the bicarbonate-stimulated adenylyl cyclase (sAC). Some acid-sensing mechanisms in other tissues, such as CAII-PDK2L1 in taste buds, might also have similar roles to play in the kidney. Finally, the function of a variety of additional membrane channels and transporters is altered by pH variations both within and outside the cell, and the expression of several metabolic enzymes are altered by acid-base status in parts of the nephron. Thus, it is possible that a master pH sensor will never be identified. Rather, the kidney seems equipped with a battery of molecules that scan the epithelial cell environment to mount a coordinated physiologic response that maintains acid-base homeostasis. This review collates current knowledge on renal acid-base sensing in the context of a whole organ sensing and response process.


A major unresolved issue in renal physiology is how renal epithelial cells sense extracellular acid-base status to initiate their homeostatic response to these stimuli. Among the regulatory factors that have been suggested are, not surprisingly, pH, CO₂, and bicarbonate as well as a number of potential hormonal stimuli.1,2 Indeed, early elegant studies by Schwartz and Al-Awqati showed that basolateral CO₂ elevation, together with an initial increase in calcium, stimulates proton secretion by proximal tubules and collecting duct intercalated cells.3,4 This occurs at least in part by inducing the apical insertion of V-ATPase in these cell types. Some candidate proteins including the Pyk2 and ErbB1/2 tyrosine kinases in the proximal tubule are also implicated in pH sensing,5–7 and a family of G-protein-coupled receptors (GPCRs) may generate cAMP or IP3/calcium signals in response to acidic pH.8 However, the sensing and signaling mechanism in renal cells remains poorly understood, and the relative contributions of apical (the tubular fluid-facing pole of the epithelial cells) versus basolateral (the interstitial fluid/blood-facing pole of the cells) sensing in the homeostatic mechanism is unclear.

ACID-BASE SENSORS ALONG THE PROXIMAL TUBULE

The Pyk2/ETB Receptor Pathway

A major site of acid-base regulation in the kidney is the proximal tubule. Here, luminal bicarbonate is reabsorbed by an indirect pathway that involves cytosolic carbonic anhydrase II (CAII) and plasma membrane–associated CAIV in collaboration with the apical membrane transporter, NHE3, an apical V-ATPase and basolateral bicarbonate transporters.9 A major acid-regulated player in this system is the sodium/proton-exchanger, NHE3. This transporter is activated at low intracellular pH, but is itself not directly stimulated by pH. The search for a signaling pathway that involves NHE3 activation led to the identification of a nonreceptor tyrosine kinases such as Pyk2 and ErbB1/2 as well as a number of potential hormonal stimuli.1,2 Indeed, early elegant studies by Schwartz and Al-Awqati showed that basolateral CO₂ elevation, together with an initial increase in calcium, stimulates proton secretion by proximal tubules and collecting duct intercalated cells.3,4 This occurs at least in part by inducing the apical insertion of V-ATPase in these cell types. Some candidate proteins including the Pyk2 and ErbB1/2 tyrosine kinases in the proximal tubule are also implicated in pH sensing,5–7 and a family of G-protein–coupled receptors (GPCRs) may generate cAMP or IP3/calcium signals in response to acidic pH.8 However, the sensing and signaling mechanism in renal cells remains poorly understood, and
kinase, Pyk2, as a potential pH sensor in proximal tubule cells. In vitro, Pyk2 is autophosphorylated and has kinase activity, which are both maximal when pH decreases within the physiologic range, from 7.4 to 7.0. The signaling cascade leading to NHE3 stimulation also depends on the activation of c-Src, which forms a complex with Pyk2.

The story is complicated by the presence of a parallel pathway of acid activation of NHE3 in which ERK1,2 and c-fos are required. ERK itself is stimulated by a decrease in pH, but this does not involve Pyk2 or c-Src. Both pathways, however, seem to converge through effects on the transcription of the endothelin receptor, ET-1, which in turn activates the ETB receptor leading to a RhoA-dependent cytoskeletal reorganization, increased NHE3 membrane accumulation, and increased proton extrusion from proximal tubule cells. Interestingly, a similar pathway leading to acid-activated citrate reabsorption by stimulation of the transporter, NaDC-1, in proximal tubule cells is also dependent on a functional endothelin B receptor.

Basolateral CO2/HCO3− Sensing in Proximal Tubules

To dissect the stimulus that regulates bicarbonate reabsorption by the proximal tubule, an elegant rapid mixing device was necessary to distinguish between the effects of HCO3−, CO2, and pH, which are in equilibrium in physiologic systems. It was shown that (at least on a short time scale) the rate of HCO3− reabsorption by the proximal tubule (JHCO3−) does not respond at all to isolated changes in basolateral pH. On the other hand, JHCO3− increases with isolated increases in basolateral CO2 and decreases with basolateral HCO3−. This effect was attributed to the activity of a receptor tyrosine kinase. Preliminary data suggest that this kinase is an ErbB1/2 heterodimer at the basolateral membrane. Moreover, additional preliminary data suggest the response to basolateral CO2 requires receptor protein tyrosine phosphatase-γ, the extracellular binding site of which is highly homologous to carbonic anhydrases.

Subsequently, it was shown that the apical angiotensin receptor, AT1A, but not the AT2 receptor, is required for the basolateral CO2 induced increase in bicarbonate reabsorption in rabbit proximal tubule S2 segments. This was achieved using pharmacological inhibition of the AT1A receptor system, and by using AT1A receptor knockout mice. Importantly, the angiotensin II effect is due to the production of endogenous ANGII peptides, and not by secretion of angiotensinogen or ANGL.

It was previously shown that basolateral CO2 stimulates V-ATPase-mediated proton secretion in isolated perfused proximal tubules and collecting duct intercalated cells by a mechanism that involves intracellular calcium elevation. Whether the sensors involved in these processes are the same as for HCO3− reabsorption remains unknown. Clearly, the sensing and signal transduction pathways in the proximal tubule are complex; further research is needed to elucidate the interaction/crossstalk (if any) between the Pyk2/ETB receptor system and the ErbB1/2 and AT1A pathway described in the preceding paragraphs.

ACID-SENSING GPCRs

Some members of the GPCR family have been identified as putative pH sensors that increase intracellular cAMP or IP3 when liganded by protons. These include GPR4, OGR1 (ovarian cancer GPCR 1), and TDAG8 (T cell death-associated gene 8). Both GPR4 and OGR1 are expressed in the kidney. Importantly, GPR4 null mice have lower serum bicarbonate and less acidic urine than wild-type mice, and do not secrete an imposed acid load as efficiently as wild-type mice. These data indicate that GPR4 is involved in acid-base sensing in the kidney. In cell culture systems, the maximal cAMP response was induced by an extracellular pH of 7.0, and the response diminished progressively at lower pH values such that cAMP generation by OMCD cells was identical at pH 7.8 and 6.6. This finding suggests that GPR4 is more likely to be involved in monitoring blood/interstitial pH rather than apical (urinary) pH in the collecting duct. The stimulatory role of cAMP in V-ATPase translocation and proton extrusion by A-type intercalated cells has already been established, implying that cAMP elevation due to activation of GPR4 by acidemia would have a similar effect.

Although it has been shown that interstitial pH in the cortex can fluctuate according to acid-base status, within the range required to activate or inactivate GPR4, the same may not be true for deeper parts of the renal medulla, where the ambient pH can be much more acidic—at least in rodents. Thus, GPR4 may be less effective as a basolateral pH sensor in the inner stripe and inner medulla.

SOLUBLE ADENYLYL CYCLASE

Over the past few years, an evolutionarily well conserved enzyme known as the soluble adenylyl cyclase (sAC) has emerged as a strong candidate to play a role in acid-base sensing in the kidney as well as in other organs and tissues. sAC is activated directly by HCO3− ions, but calcium can further modify its activity by sensitizing its response to any given bicarbonate concentration. This is not the case for sAC firmly in the spotlight as a potential sensor for monitoring extracellular acid-base status by responding to changes in intracellular bicarbonate.

sAC could also act as a CO2 sensor, because an increase in CO2 levels would produce intracellular HCO3− by the action of cytosolic carbonic anhydrases. Indeed, sAC is associated with CO2 sensing in fungi and bacteria. Despite its name, sAC is present in both soluble and particulate cellular fractions and is thought to mediate cAMP-dependent processes at various intracellular locations, including mitochondria, nuclei, and centrioles. sAC is expressed in many kidney tubules, including proximal tubules, thick ascending limbs of Henle, distal convoluted tubules, and collecting ducts, in which high levels of
V-ATPase are also found.31,32 These findings are consistent with the HCO$_3^-$-stimulated adenyl cyclase activity previously described in rat kidney.33 cAMP directly stimulates V-ATPase membrane accumulation and proton secretion by A-type intercalated cells in the kidney (Figure 1),33 and the stimulatory effect of HCO$_3^-$ on V-ATPase translocation in tissue slices in vitro is inhibited by incubation with the sAC inhibitor, H$7$.22 That sAC-mediated cAMP signaling may constitute a general sensing mechanism for regulating V-ATPase–mediated proton transport is supported by observations from other proton transporting tissues including the epididymis31 and the teleost intestine34 and teleost gill.35 In all of these tissues, sAC is involved in the regulation of V-ATPase accumulation at the cell surface in response to extracellular HCO$_3^-$ (Figure 1).

Importantly, sAC co-immunoprecipitates with the V-ATPase and these proteins are colocalized in both A- and B-type intercalated cells.32 This raises the possibility that localized signal transduction by HCO$_3^-$ and cAMP occurs at the level of the V-ATPase complex to regulate proton secretion through enzyme mobilization and possibly activity. The electroneutral sodium bicarbonate transporter, NBC3, also associates with the V-ATPase in intercalated cells,36 supporting the existence of a HCO$_3^-$–regulated signaling complex involving the V-ATPase. The production of cAMP by sAC has been discussed in relation to the generation of specific signals within restricted areas of the cell rather than throughout the cytosol as a whole.37,38 The environment around the V-ATPase contains regulatory proteins that form a microcomplex in which such signaling can occur. cAMP activates protein kinase A and the exchange proteins Epac 1 and 2, which are guanine nucleotide-exchange factors for the small GTPases, Rap1 and Rap2.39 After being activated by cAMP, Epac increases its exchange activity toward Rap1 and, in turn, GTP-bound Rap1 activates downstream effectors. Interestingly, activation of Rap2B by Epacs stimulates phospholipase C, which increases intracellular calcium,40 which is required for membrane accumulation of V-ATPase.4

**V-ATPase as a pH Sensor**

In the search for pH-sensing proteins, a relatively recent and unexpected finding is that the V-ATPase itself can serve this function in endosomes. This discovery was made using the receptor-mediated albumin degradative pathway in the proximal tubule as a model system.41 For many years, inhibition of V-ATPase–dependent endosomal acidification has been linked to abnormal intracellular protein/vesicle trafficking,42–44 resulting in epithelial dysfunction that can lead to disorders such as renal Fanconi syndrome—a proximal tubule defect.45 Whereas the effect of increasing vesicle pH on the function of some organelles, including lysosomes, is due to an alteration of the function of proteins contained within the organelle (hydrolytic enzymes), the mechanism(s) by which changes in endosomal luminal pH lead to impaired vesicle trafficking are unclear. Initial observations showed that endosomal acidification leads to the recruitment of cytosolic proteins, including small GTPases and their regulatory proteins as well as the coat protein β-COP,46 to the cytosolic side of their limiting membrane. Importantly, the transmembrane a$_2$ subunit isoform of the V-ATPase, which is targeted to early endosomes in the proximal tubule, directly interacts with a guanine-nucleotide exchange factor, ARNO, and is involved in this pH-dependent recruitment process.41 The GTPase Arf6, in contrast, interacts with a different component of the V-ATPase complex—the small c-subunit—which is part of the membrane-associated V$_0$ sector of the V-ATPase. The V-ATPase thus has a novel function as an endosomal pH sensor, possibly through one or more of the histidine residues that are located in extracellular (intraendosomal) loops of the a$_2$ subunit.47 The acidification-dependent interaction between V-ATPase subunits and small GTPases is crucial for protein trafficking between early and late endosomes,41 presumably through the recruitment of coat proteins that direct vesicle sorting within the cell. Thus, although not strictly speaking a sensor of extracellular pH, the V-ATPase itself plays a major role in intracellular pH sensing in the proximal tubule, which affects trafficking along the critical protein degradative pathway in these cells.

**Acid-base Regulation of Proximal Tubule Metabolism**

Although the specific sensor remains unclear, acidosis activates metabolic processes in the proximal tubule that lead to increased synthesis of HCO$_3^-$ and NH$_4^+$. This involves a pH-induced increase in many enzymes and transporters involved in glutamine catabolism and a resulting increase in HCO$_3^-$ and NH$_4^+$ production and transport. These transporters and enzymes include the SNAT3 glutamine transporter, glutaminase, glutamate dehydrogenase, α-ketoglutarate dehydrogenase, and phosphoenolpyruvate carboxykinase (PEPCK).48 This response is at least in part due to enhanced stability and transcription of mRNAs of the respective proteins. In addition, the RNAs encoding many other proteins in proximal tubular cells are either increased or decreased various times after onset of metabolic acidosis, and protein levels themselves show acute changes in some cases (PEPCK is significantly elevated just 1 hour after gastric gavage of NH$_4$Cl in rats), indicating a complex and varied response of this tubular segment to acid-base perturbation. Stabilization of mRNA involves an AU-repeat serving as a pH-responsive element binding ζ-crystallin, AUF-1, and HuR.49 At least in the case of PEPCK, HuR must bind to the pH-responsive element on the 3′-UTR of the mRNA encoding PEPCK to increase the $t_{1/2}$ of its mRNA.50 A similar mechanism involving ζ-crystallin has been proposed to enhance mRNA and protein expression of the loop-diuretic-sensitive NKCC2 cotransporter in the TAL.51–53 Whether any of the pH sensors described above are involved in these processes is unknown.
INSULIN RECEPTOR–RELATED RECEPTOR AS AN ALKALI SENSOR

The insulin receptor–related receptor (InsR-RR) belongs to the family of the insulin receptor and related receptors. It acts as a receptor tyrosine kinase but is not activated by insulin or IGF1. In addition to the kidney, the InsR-RR is expressed in a few other tissues including pancreas, stomach, and brain. In the kidney, the InsR-RR is exclusively found at the basolateral side of non-type A intercalated cells (bicarbonate-secretory B-IC and so-called non-A/non-B-IC). Activation of the InsR-RR occurs at levels of pH more alkaline than 7.8 and is independent from buffer media composition. In vitro, stimulation of the InsR-RR leads to autophosphorylation and subsequent phosphorylation of ERK1/2 with possible rearrangements of the actin cytoskeleton. In vivo, the InsR-RR may be involved in triggering the bicarbonate-secretory response of B-IC during alkalosis; InsR-RR–deficient mice become more alkalotic during bicarbonate loading and express lower levels of pendrin, the rate-limiting bicarbonate exchanger of B-IC.

INDIVIDUAL pH-SENSITIVE PROTEINS

In discussions regarding the pH sensing process in the kidney, one must also take into account the many proteins whose activity is modulated by pH changes. While not strictly speaking pH sensors (which generate an intracellular signaling cascade in response to pH), the effect of pH on channel activity can certainly affect cell function in a way that contributes to the overall pH response. Ion channels from different protein families exhibit an exquisite sensitivity to alterations in either intracellular or extracellular proton concentrations. Some aquaporins, including AQP0, AQP3, AQP4, and AQP6, are regulated by pH through its effect on extracellular histidines. Several potassium channels are altered in their activity by intracellular or extracellular protons. Examples include the ROMK and TASK channels. The renal outer medullary K channel, ROMK (Kir1.1), responsible for renal potassium excretion along the thick ascending limb and collecting duct, is inhibited by intracellular protons possibly acting on hydrophobic leucines at the cytoplasmic face of the inner transmembrane helices. The direct inhibitory effect of pH on ROMK gating contributes to reduced renal K clearance during acute acidosis.
TASK K channels (TWIK-related acid-sensitive K+ channels) are closed by protons and activated by extracellular alkalization/HCO_3^- and may be involved in acid/CO_2 sensing in different organs. In kidney proximal tubules, TASK2 channels are stimulated by HCO_3^- fluxes across the basolateral membrane and thereby stabilize the membrane potential necessary for driving solute transport in this nephron segment. ASC channels and ENaC channels formed by the αβγ subunits are gated by extracellular protons. These channels may play a role in pain sensation and sour taste in extrarenal tissues, but no role in renal function has been reported to date. Similarly, members of the transient receptor potential (TRP) ion channel family are also very sensitive to extracellular protons, including TRPV1 and PDK2L1. TRPV1 is important in pain sensation, whereas PDK2L1 mediates at least in part the taste of sour and, in cooperation with CAIV, the taste of carbonation in tongue taste buds. PDK2L1 is highly expressed in the kidney and closely related to the ADPKD-causing polycystins, but no role of PDK2L1 in the kidney has been described at present.

Another candidate for a signaling pH sensor is the calcium-sensing receptor. The external cellular pH modulates the response of this receptor to both Ca^{2+} and Mg^{2+}, increasing sensitivity at acidic pH and decreasing sensitivity at alkaline pH at least in cultured kidney 293 cells. This may occur through the modulation of electrostatic interactions within the cation-binding pocket of the receptor. Whether this effect is relevant in vivo remains to be confirmed. A similar mechanism may be responsible for the pH sensitivity of L-type calcium channels, in which protonation of a well-defined cluster of glutamine residues blocks channel activity.

**CLINICAL RELEVANCE FOR RENAL pH SENSING**

In addition to an important role in defending systemic acid-base homeostasis, renal acid-base sensing may have additional clinical implications. Progressive loss of kidney function in CKD is paralleled by the development of metabolic acidosis, an additional and independent risk factor for morbidity and mortality. Recent studies in several smaller cohorts of patients with stage 2–3 CKD and in animal models suggest that acidosis promotes the further progression of renal insufficiency. The mechanism may involve the activation of the complement system on one hand and an endothelin and aldosterone-dependent mechanism on the other. However, it remains to be established which processes in the progression of CKD are stimulated by acidosis, and how alkali supplementation slows its progression. It is also not clear why chronic acidosis in the setting of inborn forms of renal tubular acidosis does not lead to renal insufficiency, suggesting that acidosis alone is not sufficient to drive disease progression.

The kidney is an exquisitely pH-sensitive organ that is able to regulate the function of specific processes in response to acute and chronic changes in systemic and local pH. Distinct proteins have been identified that are capable of sensing intracellular or extracellular changes in pH and bicarbonate concentration. However, the exact role of most putative sensors in vivo remains to be established. It is becoming clear that none of these sensors is a master regulator; rather, these sensors probably generate a complex network of signals that act together to fine-tune the response of the kidney (and other organs) to acid-base changes and work to maintain a constant environment that is critical for vital functions of cells and organs in the body.

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**DISCLOSURES**

None.

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