

Acid sensing in renal epithelial cells

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J Clin Invest. 2004;114(12):1696-1699. <https://doi.org/10.1172/JCI23864>.

Commentary

The kidney adjusts net acid excretion to match production with exquisite precision, despite little or no change in the plasma bicarbonate concentration. The acid-sensing pathway that signals the kidney to increase acid secretion involves activation of the proto-oncogene c-Src. A new study in this issue shows that proline-rich tyrosine kinase 2 (Pyk2) is responsible for acid-induced activation of c-Src and is essential for acid sensing in renal epithelial cells. The findings implicate a broader role for Pyk2 in acid-base homeostasis in bone and other tissues beyond the kidney.

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Acid sensing in renal epithelial cells

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The kidney adjusts net acid excretion to match production with exquisite precision, despite little or no change in the plasma bicarbonate concentration. The acid-sensing pathway that signals the kidney to increase acid secretion involves activation of the proto-oncogene c-Src. A new study in this issue shows that proline-rich tyrosine kinase 2 (Pyk2) is responsible for acid-induced activation of c-Src and is essential for acid sensing in renal epithelial cells (see the related article beginning on page 1782). The findings implicate a broader role for Pyk2 in acid-base homeostasis in bone and other tissues beyond the kidney.

Although the principal product of metabolism in mammalian cells is the volatile acid carbon dioxide, humans on a typical Western diet produce about 70 millimoles of nonvolatile acid per day. Remarkably, varying metabolic acid production over a range of 0–150 millimoles is accompanied by a matching increase in net acid excretion by the kidney with a change of only 1 mM in plasma bicarbonate concentration (1). The adaptive responses that enable the kidney to increase net acid excretion in response to

increased acid generation have been studied extensively in animal models of metabolic acidosis. In the proximal tubule, acidosis increases the activity of luminal and basolateral proteins involved in bicarbonate transport (2, 3), ammonia generation (4), and the reabsorption and metabolism of citrate (5). In the collecting duct, acidosis suppresses bicarbonate secretion (6) and stimulates recruitment of proton pumps to the luminal membrane of intercalated cells (7). Of the acid-base transporters in the proximal tubule, the luminal sodium/hydrogen exchanger 3 (NHE3) has a prominent role, and the mechanism by which its activity increases during metabolic acidosis has been examined in some detail. Metabolic acidosis acutely increases the kinetic activity of NHE3 through direct pH effects and by phosphorylation (8), while chronic

acidosis increases the number of NHE3 transporters (9).

Acid-base transporter kinetics cannot account for precise pH sensing

How does the kidney “know” to adjust net acid excretion with such precision with only minimal changes in plasma bicarbonate concentration? Available data in the physiology literature suggests that transporter kinetics alone cannot account for this degree of sensitivity. In the proximal tubule, a reduction in extracellular bicarbonate induces a fall in intracellular pH, which directly activates the sodium/hydrogen exchanger through an intracellular pH regulatory site (10). This requires a change in intracellular pH of about 0.1 to achieve a 50% increase in the rate of transport or an approximately 5% change in the rate of transport in response to a change in extracellular bicarbonate concentration of 1 mM. Both the luminal vacuolar H⁺-ATPase and the basolateral sodium bicarbonate cotransporter in the proximal tubule are even less responsive to changes in intracellular pH (11–13). This suggests that a bicarbonate (or pH) sensor that can amplify luminal proton secretion must be present.

Nonstandard abbreviations used: FAK, focal adhesion kinase; NHE3, sodium/hydrogen exchanger 3; OKP, opossum kidney clone P; Pyk2, proline-rich tyrosine kinase 2.

Conflict of interest: The author has declared that no conflict of interest exists.

Citation for this article: *J. Clin. Invest.* **114**:1696–1699 (2004). doi:10.1172/JCI200423864.

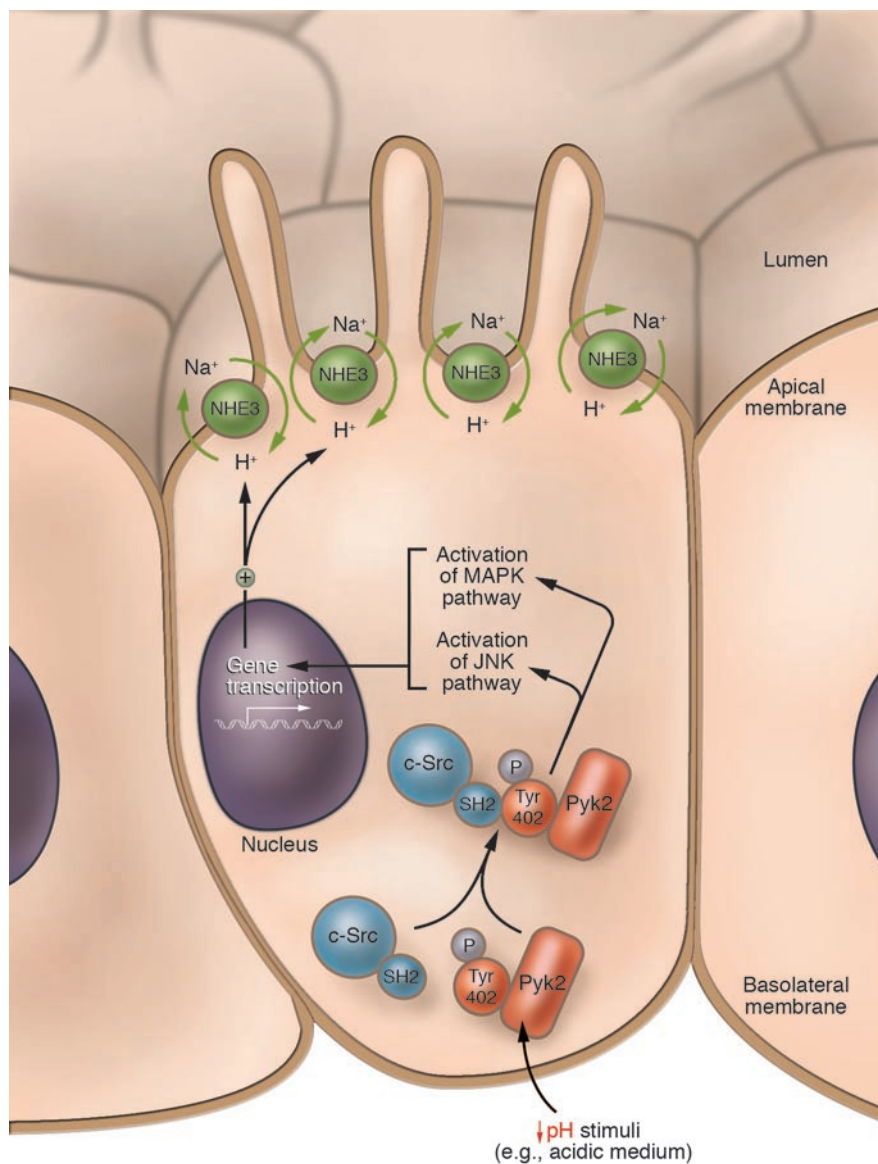


Figure 1

Acid-sensing pathway in renal proximal tubular cells. A drop in extracellular fluid pH induces a corresponding decrease in intracellular pH that induces activation of Pyk2, through an unidentified mechanism, by phosphorylation on tyrosine 402. Phosphorylated Pyk2 binds to the SH2 domain of c-Src, phosphorylating and activating it, producing subsequent activation of the MAPK and JNK signaling pathways and an increase in transcription of NHE3, the sodium-hydrogen exchanger of the proximal tubule brush border.

Activation of NHE3 by cytosolic acidification requires c-Src phosphorylation

In this issue of the *JCI*, Li, Sato, and colleagues (14) have attempted to identify the kidney's elusive pH sensor. These authors used cultured opossum kidney clone P (OKP) proximal tubule cells exposed to acid media as an in vitro model of the renal adaptation to acidosis. They found that a 24-hour exposure to acid increased NHE3

activity and protein abundance (15). They also found that the increase in NHE3 activity required tyrosine kinases (16). A subsequent study identified the proto-oncogene *c-Src* as the cellular tyrosine kinase essential for this response (17). Activation of *c-Src* tyrosine kinase (herein referred to as c-Src) requires phosphorylation on an internal tyrosine residue, and changes in extracellular pH of 0.07 (equivalent to a change in bicarbonate concentration of

3–4 mM) were found to induce a detectable increase in phosphorylation of c-Src (18). Surprisingly, the phosphorylation of c-Src was greatest after only 90 seconds of cellular acidification, subsequently returning to a level slightly above baseline. This observation prompted the investigators to seek a protein that could sense small changes in cytosolic pH and induce c-Src phosphorylation within 90 seconds.

Pyk2 is an activator of c-Src and candidate pH sensor

In the present study (14), Li, Sato and colleagues make the case that the proline-rich tyrosine kinase 2 (Pyk2), a member of the focal adhesion kinase (FAK) family, acts as both a pH sensor and activator of c-Src. Pyk2, a 116-kDa cytoplasmic protein tyrosine kinase, is activated by phosphorylation on tyrosine residues in response to various stimuli, depending on the cell type, including growth factor receptors, chemokine receptors, G protein-coupled receptors, osmotic stress, cell depolarization, and others (19, 20) (Figure 1). For most of these stimuli, activation of Pyk2 requires intracellular calcium release (19). In contrast to FAK, which is localized to adhesion plaques at the basal side of the cell, Pyk2 is located in the cytosol but can be recruited to plasma membrane, the perinuclear region, or the nucleus in response to different stimuli (20). Phosphorylation of tyrosine 402 on Pyk2 induces the formation of a complex with the SH2 domain on c-Src (21), leading to activation of MAPK and JNK signaling pathways (21, 22).

Li, Sato, et al. (14) show that Pyk2 is rapidly phosphorylated following exposure of renal epithelial cells to an acidic medium, with peak phosphorylation occurring at 30 seconds after exposure and followed by a persistent low level of increased phosphorylation. Expression of a catalytically inactive dominant-negative Pyk2 prevented the acid-induced activation of NHE3 but had no effect on glucocorticoid-stimulated NHE3 activation. Similarly, suppression of Pyk2 protein by the transfection of cells with a small interfering RNA specific to the opossum mRNA inhibited acid-induced activation of NHE3 without affecting activation by glucocorticoids.

The authors showed that Pyk2 kinase activity and its binding to c-Src are essential for acid-induced c-Src activation. Acid incubation increased the amount of c-Src binding to Pyk2 (14). Further, in cells expressing the mutant Pyk2Y402F, which was gener-



ated to eliminate the c-Src binding site, acid incubation produced no significant activation of NHE3. Finally, expression of a dominant-negative kinase-inactive Pyk2 prevented acid incubation from increasing the tyrosine kinase activity of c-Src.

Then how does the renal epithelial cell sense acid? The authors propose that Pyk2 itself is the sensor (14). They found that the kinase activity of Pyk2 *in vitro* is pH dependent, with a 3-fold increase in kinase activity and a nearly 2-fold increase in autophosphorylation activity at pH 7.0 compared with the normal intracellular pH, 7.2. But the devil is in the details. The *in vitro* experiments were performed at an ATP concentration of 10 μ M. The principal effect of pH was to shift the K_m for ATP from 129 μ M to 51 μ M, concentrations that are both far below the cytosolic ATP concentration. When kinase activity was measured at higher ATP concentrations, the decrease in pH had no effect. Li, Sato, et al. propose the interesting suggestion that the pH dependence of kinase activity may still be physiologically relevant, citing studies by Mandel and colleagues (23) showing that for the proximal tubule Na,K-ATPase, the apparent K_m for cellular ATP concentration in the intact tubule was much higher than the K_m for ATP concentration of the isolated enzyme. The studies by Mandel et al. are probably not comparable, however, since the K_m for ATP in the Na,K-ATPase is 10-fold higher than that for Pyk2 and because the inhibitors used to alter cellular ATP levels (23) could have affected Na,K-ATPase activity indirectly.

Second, the authors showed that acid activation of Pyk2 *in vitro* was inhibited by EGTA, which suggests calcium dependence, although their prior work showed no effect of acid incubation on cell calcium and no effect of the calcium buffer BAPTA (24), which prevents increases in cell calcium, on acid-induced immediate-early gene expression (16). Last, experiments in which Pyk2 was overexpressed showed no increase in basal NHE3 activity, as might be expected for a pathway sensitive to small changes in Pyk2 activity. So, although the studies provide compelling evidence that Pyk2 is crucial for the acid-sensing pathway, the elusive acid sensor remains a mystery.

Importance of Pyk2 in acid-base homeostasis beyond the kidney

Pyk2 is involved not only in luminal acid secretion, but also in basolateral bicarbonate exit in the proximal tubule (25). The findings of this study (14) have important implica-

tions for acid sensing in other cells beyond the kidney. A surprising phenotype of the c-Src-knockout mouse is osteopetrosis (26), a disorder of inadequate osteoclast bone resorption. In other cell types, other Src family kinases such as Fyn and Yes probably compensate for c-Src deficiency (27). Osteoclasts respond to even subtle metabolic acidosis by secreting acid, which, rather than being excreted in urine, is buffered by alkaline bone salts (28). Recent studies show that Pyk2 binding to c-Src is essential for integrin-mediated osteoclast activation, although unlike kidney cells in the present study, osteoclast activation was not effected by expression of a kinase-inactive Pyk2 (29).

An important physiologic consequence of extracellular fluid acidification is release of endothelin from renal endothelium (30). Endothelin stimulates acid secretion in both the proximal tubule and collecting duct (31, 32). Pyk2 has a prominent role in the response of endothelial cells to a variety of mechanical, hormonal, and other stimuli (33). In cardiomyocytes Pyk2 is pivotal for integrin-mediated release of endothelin (34). It will be interesting to determine whether the acid-sensing pathway for endothelin release in endothelial cells is also Pyk2 dependent and what similarities this pathway shares with renal epithelial cells. It would be equally interesting to determine whether activation of endothelium through this pathway by mild or subclinical acidosis plays a role in the greatly increased risk of cardiovascular mortality found in patients with chronic kidney disease (35).

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TB, or not TB: that is the question — does TLR signaling hold the answer?

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Innate immunity critically depends on signaling by Toll-like receptors (TLRs) that rely heavily on an intracellular adapter protein called myeloid differentiation factor 88 (MyD88). Adaptive immune defenses are generally thought to be orchestrated by innate immune responses and so should require intact TLR-MyD88 signaling pathways. But a surprising new study in MyD88-null mice infected with *Mycobacterium tuberculosis* challenges this view and instead suggests that MyD88 may not be absolutely required for a normal adaptive immune response (see the related article beginning on page 1790).

Every second of every day, *Mycobacterium tuberculosis* (MTB) infects another human being somewhere in the world (1), yet the global incidence of around 8 million cases of active tuberculosis (TB) per year is only a tiny fraction of the estimated 2 billion people infected. Only around 1 in 10 of those infected will ever develop active disease, which raises a question: why do some individuals develop disease, while others do not? The answer is not trivial: in 2002, TB caused an estimated 2 million deaths, mostly in underdeveloped countries, and over the last century, TB has killed an estimated 100 million people (1, 2). A solid mechanistic understanding of how MTB infection proceeds and how host defenses are marshaled against it (both successfully and unsuccessfully) has proven elusive and remains an important goal.

Nonstandard abbreviations used: IRF, IFN-regulated factor; MTB, *Mycobacterium tuberculosis*; MyD88, myeloid differentiation factor 88; NOD, nucleotide-binding oligomerization domain; TB, tuberculosis; TIR, Toll/IL-1 receptor resistance; TLR, Toll-like receptor; TRAM, TRIF-related adapter molecule; TRIF, TIR domain-containing adapter inducing IFN- β .

Conflict of interest: The authors have declared that no conflict of interest exists.

Citation for this article: *J. Clin. Invest.* **114**:1699–1703 (2004). doi:10.1172/JCI200423867.

In this issue of the *JCI*, Fremond and coworkers report data that expand our understanding of the host defense against *M. tuberculosis* infection yet raises provocative new questions that will be sure to spark controversy (3). They used mice with a genetic deficiency in myeloid differentiation factor 88 (MyD88), a critical adapter molecule common to signaling by most Toll-like receptors (TLRs). MyD88^{-/-} mice were subject to infectious challenge with MTB and died within 4 weeks, showing evidence of massive, uncontrolled pulmonary growth of the infectious organism. Adaptive immune responses appeared to be unaffected, but iNOS activation and expression of the defensive cytokines TNF- α , IL-6, and IL-12 p40 were strongly reduced in MyD88^{-/-} macrophages and DCs. Analyses of infected lung tissues revealed massive infiltration of mononuclear cells and neutrophils in infected MyD88-null mice, as might be expected, but flow cytometric studies showed no differences in CD4⁺ and CD8⁺ T cell recruitment to lungs between infected MyD88-null and wild-type mice. These results are consistent with a normal adaptive immune response and a robust inflammatory response

with increased neutrophil and macrophage recruitment in MyD88-null mice yet markedly blunted defense by innate mechanisms that eventually proved lethal. Perhaps the most intriguing finding was that vaccination of MyD88-null mice with *Mycobacterium bovis* BCG induced appropriate activation of T cells and induction of a Th1 response to mycobacterial antigens, but this only forestalled death, despite the fact that induction of cytokines (IL-1 β , IFN- γ , TNF- α , MIP-1 α , and MCP-1) in the lungs did not differ between MyD88-null mice and wild-type controls. The authors concluded that MyD88-dependent signaling is not significantly involved in T cell activation but that in the absence of MyD88, T cell-mediated immunity can afford only partial protection from infection. At face value, this work would appear to offer a plausible answer to the question posed — TB, or not TB? — namely, that innate immunity in general and TLR signaling through MyD88 are essential for the host to take arms against the sea of troubles that MTB infection brings. But how does this answer square with what we know about the interaction between innate and adaptive immunity?

Signaling by Toll-like receptors and MyD88 in innate immunity

Defense against invading pathogens comes in 2 major forms. Innate immunity rapidly mounts a multipronged defense that is relatively nonspecific and relies heavily on inflammatory molecules directly or indirectly toxic to pathogens. Adaptive