Connecting type A intercalated cell metabolic state to V-ATPase function: phosphorylation does matter!

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THE V-ATPASE IS A LARGE (~900 kDa) multisubunit enzyme that utilizes energy derived from hydrolysis of ATP to translocate H+ across biological membranes. In the kidney, apical V-ATPase regulates H+ secretion into the urine and there is evidence for its existence in the proximal tubule, thick ascending limb, distal convoluted tubule, connecting tubule, and collecting duct (4, 17). The H+ secretory capacity of the distal tubule, connecting tubule, and collecting duct is much smaller than that of the proximal tubule; however, and as true for other ions, the final adjustment of the urinary H+ concentration is established here. The V-ATPase consists of a transmembrane V0 domain with subunits a, c, and d (several copies of the c subunit), and a catalytic V1 domain with subunits A–H. The versatility of this proton pump is increased by the fact that some subunits have several isoforms, and subunit composition can vary considerably depending on the site of expression. The functional importance of some of these subunits has come from genetic studies in humans and gene-targeted mice. In humans, mutations in the B1 or a4 subunit are associated with distal renal tubular acidosis (9). Patients with a4 mutations also exhibit perturbed proximal tubule function. In contrast to the findings in humans, B1 subunit knockout mice did not display overt metabolic acidosis at baseline (5). However, when subjected to an acid load, the B1 knockout mice exhibited a defect in urine acidification compared with wild-type mice. Importantly, it was found that the B2 subunit was able to substitute for B1 in these knockout mice, allowing for compensation under baseline conditions (13). On the other hand, a4 subunit knockout mice suffer not only from severe acidosis but also from proximal tubule dysfunction with defective endocytic trafficking, proteinuria, phosphaturia, and accumulation of lysosomal material (7).

Despite the functional significance of this pump, not much is known about the regulation of V-ATPase function. Soluble adenyl cyclase (sAC)-mediated cAMP signaling is thought to be a sensing mechanism (possibly via pH, CO2/HCO3-) for V-ATPase-mediated H+ transport (11, 12), a finding that sheds light on how extracellular acid-base status is sensed by renal epithelial cells. Furthermore, recent studies indicate that the apical (pro)renin receptor of the collecting duct, a receptor activated by renin and prorenin, has a functional interrelationship with V-ATPase (1), possibly connecting the renin-angiotensin-aldosterone system to acid-base homeostasis (18, 19). In addition, although not much is known about the molecular mechanisms, it is clear that increasing flow can stimulate V-ATPase activity (10).

In a recent issue of the American Journal of Physiology-Renal Physiology, Alzamora et al. (2) provide novel insights into the regulation of V-ATPase, linking cellular energy homeostasis to V-ATPase function via 5’ adenosine monophosphate-activated protein kinase (AMPK)-dependent phosphorylation of the V-ATPase A subunit (ATP6V1A). In this study, the authors demonstrated in isolated, perfused outer medullary collecting ducts that V-ATPase function can be inhibited by 60-min preincubation with 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR), an AMPK activator. Furthermore, the authors identified that serine residue 384 on the V-ATPase A subunit is a targeted phosphorylation site for AMPK by utilizing liquid chromatography and matrix-assisted laser desorption/ionization mass spectrometry. This novel finding was confirmed in HEK-293 cells stably transfected with constructs for doxycycline-inducible AMPK knockdown. Interestingly, site mutagenesis studies revealed that HEK-293 cells expressing an AMPK phosphorylation-deficient serine 384 mutant acidified extracellular pH more compared with the wild-type A subunit, indicating a tonic inhibitory role of this phosphorylation site for H+ secretion. Finally, the authors showed that in rabbit clone C cells (intercalated cells), AMPK-dependent serine 384 phosphorylation regulates both V-ATPase activity and cytosolic subcellular redistribution.

Despite the many strengths of this study, a number of questions remain unanswered. The authors have previously shown that AMPK and protein kinase A (PKA) have opposing effects on subcellular localization and activity of the V-ATPase in kidney intercalated cells. In the present study, the authors discovered a targeted phosphorylation site for AMPK on the A subunit; however, PKA activity had to be blocked to unravel this finding and it is still not clear how PKA, by phosphorylating serine 175 of the A subunit (3), functionally antagonizes the effects of serine 384 phosphorylation. Their future studies will be important in clarifying the interactions between these two pathways and may elucidate how PKA-dependent phosphorylation of serine 175 can couple the sensing of acid-base status to pump function while AMPK-dependent phosphorylation of serine 384 can couple V-ATPase activity to metabolic stress. An alternative strategy could be to study V-ATPase phosphorylation by a phosphoproteomics approach (8). Combining this method with a recently published fluorescence-activated cell sorting of intercalated cells (16) could give the opportunity to simultaneously identify other phosphorylation sites under unstimulated and stimulated conditions in a cell type-specific manner.

It would be interesting to expand this knowledge and study other parts of the nephron to determine whether there is a cortical-medullary difference in the regulation of V-ATPase, a situation found for several other proteins including adenyl cyclase 6-mediated Na-K-2 CI cotransporter and aquaporin-2...
phosphorylation after vasopressin stimulation (14, 15). It seems reasonable to assume that such a region-specific regulation also exists for V-ATPase, which could further increase the regulatory versatility of V-ATPase-mediated H⁺ secretion along the nephron. In the proximal tubule, in vivo micropuncture studies employing bafilomycin (V-ATPase inhibitor) added to the luminal perfusion solution showed a ~50% reduction in H⁺ secretion (6); however, comparable in vivo experiments studying the role of AMPK for urinary acidification in further distal segments are still missing.

Last, it would be interesting to address the question of how AMPK-mediated inhibition of H⁺ excretion regulates the milieu intérieur. It is commonly accepted that hypoxia triggers acidosis, which would indeed require increased H⁺ secretion via the kidneys, possibly via V-ATPase. However, hypoxia is also known to activate AMPK, so the finding that AMPK is inhibiting H⁺ secretion is quite the opposite from what is expected to be physiologically necessary. On the other hand, saving ATP in conditions of low energy supply by inhibiting active H⁺ secretion via V-ATPase could prevent detrimental ATP depletion of intercalated cells during such conditions. Taken together, Alzamora et al. (2) have convincingly demonstrated that phosphorylation of the A subunit at serine 384 by AMPK plays a key role in inhibition of V-ATPase-mediated H⁺ secretion in the kidney, thus taking us one step closer in linking V-ATPase function and metabolic status.

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