The ugly duckling of urinary acidification: what is the contribution of the thick ascending limb of the loop of Henle to urinary acidification?

Carsten A. Wagner,1 Nilufar Mohebbi,2 and Soline Bourgeois1
1Institute of Physiology, University of Zurich, Zurich, Switzerland; and 2Division of Nephrology, University Hospital Zurich, Zurich, Switzerland

THE KIDNEY EXCRETES ACID in the form of titratable acidity and ammonium as well as in the form of free protons. The latter causes urinary acidification together with the reabsorption of filtered bicarbonate. Inborn or acquired forms of renal tubular acidosis (RTA) reduce the capacity of the kidneys to form ammonium and excrete acids (9). According to the functional defect and predominant site of renal damage, different subtypes of RTA have been classified: type I or distal RTA (dRTA), describing defects localized in the connecting tubule and collecting duct; type II or proximal RTA, caused by defects in the proximal tubule, and type IV or hyperkalemic RTA, summarizing different causes of relative aldosterone insufficiency or resistance in the distal nephron. Some authors use also the term type III RTA or combined RTA, referring to a mixed proximal and distal nephron pathology. Clinically, the distinction of these RTA subtypes depends on the detection of normal anion gap metabolic acidosis for all subtypes combined with bicarbonaturia or other signs of proximal tubule dysfunction (Fanconi syndrome) for type II proximal RTA or inappropriately alkaline urine in the face of metabolic acidosis for type I dRTA and the presence of hyperkalemia for type IV RTA.

However, this distinction may not always be as obvious as it seems as compensatory mechanisms may mask overt metabolic acidosis or only partial defects may be present. Thus, specific provocation tests challenging the kidneys’ capability to reabsorb bicarbonate or to produce maximally acidic urine are routinely used to unmask such defects. To date, two types of urinary acidification tests are in clinical use: the ammonium chloride loading test and the furosemide-fludrocortisone (F test) may be used, combining a mineralocorticoid such as fludrocortisone with a loop diuretic such as furosemide. This test was first introduced as a single application of furosemide by D. Batlle (1) aiming to provide pathophysiological insights and distinction into different causes of type I dRTA. It was further refined and validated by Walsh and colleagues (19) by adding a mineralocorticoid (e.g., fludrocortisone). The assumption underlying this test is that electrogenic Na+ absorption via the epithelial Na+ channel (ENaC) in principal cells in the connecting tubule and collecting duct causes a lumen negative potential that would enhance proton secretion by neighboring type A intercalated cells acidifying urine. The application of a mineralocorticoid increases ENaC activity, and the loop diuretic causes a shift in Na+ reabsorption from the thick ascending limb of the loop of Henle (TAL) to the connecting tubule and collecting duct, the major site of ENaC expression. This test is well tolerated by patients and appears to be also similarly reliable as the ammonium chloride loading test. However, new data presented in the American Journal of Physiology-Renal Physiology by de Bruijn and colleagues (5) challenge the interpretation of this test and ask the question of whether the TAL plays a much more important and hitherto underestimated role in urinary acidification.

de Bruijn et al. report that furosemide not only blocks Na+–K+–2Cl− cotransporter 2 (NKCC2) in TAL cells but also stimulates Na+/H+ exchanger (NHE)3. The reduced absorption of Na+ via NKCC2 increases the Na+ gradient, providing a stronger driving force for NHE3 activity. Consequently, higher NHE3 activity would excrete more protons and acidify urine. These observations made in microperfusion experiments were further translated to the whole animal situation in technically very refined clearance experiments in mice by measuring in real-time furosemide-induced urinary acidification in the absence and presence of a specific NHE3 inhibitor. As expected, the absence of the NHE3 inhibitor, furosemide caused a fall in urinary pH and an increase in the excretion of ammonium, whereas in the presence of the NHE3 inhibitor, urinary acidification and ammonium excretion were much reduced. Based on these data, the authors suggest that the TAL contributes to urinary acidification and that abnormal urinary acidification in response to furosemide may not only reflect a pathology in the connecting tubule and collecting duct (5).

The TAL contributes to renal acid excretion by at least two processes: filtered bicarbonate, not reabsorbed by the proximal tubule, is mostly reabsorbed by the TAL involving NHE3 and V-type H+–APases localized in the luminal membrane and a Cl−/HCO3− exchanger together with a K+–HCO3− cotransporter at the basolateral membrane (2, 4). Second, the TAL reabsorbs a major fraction of ammonium secreted by the proximal tubule into urine and accumulates ammonium in the medullary interstitium. This process involves NKCC2 accepting NH4+ instead of K+ and basolateral NHE4 and Na+–HCO3− cotransporter 1 involved in the basolateral release of ammonium (3, 11, 15, 18).

Seminal work by Ullrich and Eigler (17) and Gottschalk et al. (8) in the late 1950s and early 1960s examined the sites of urinary acidification using micropuncture in rats and hamsters. They demonstrated that urine passing along the proximal
tubule is acidified from pH 7.4 to pH ~6.8 due to the removal of bicarbonate. Subsequently, urine pH remained fairly stable until the next site accessible to micropuncture, the distal convoluted tubule, whereas urine collected at the tip of the renal medulla was much more acidic. From these data, it was concluded that the collecting duct system was the major site of urinary acidification.

So what speaks against or in favor of the “collecting duct” interpretation of the $F+F$ test? A study (10) in rats showed that the ENaC blockers amiloride and triamterene reduce the furosemide-induced urinary acidification. However, amiloride at higher concentrations also blocks NHE3. Inborn forms of dRTA are caused by mutations in the ATP6V1B1 (B1) and ATP6V0A4 (a4) subunits of V-type H+-ATPase and in anion exchanger 1 (SLC4A1). In these patients, the $F+F$ test causes less urinary acidification. Of note, at least ATP6V1B1 and ATP6V0A4 are not only expressed in intercalated cells along the connecting tubule and collecting duct but also in the TAL (14, 16). In mice lacking the ATP6V1B1 B1 subunit, furosemide causes less urinary acidification than in wild-type mice. Moreover, inhibition of ENaC with benzamil or amiloride similarly reduced urinary acidification. Mice lacking the $\alpha$-subunit of ENaC in the cortical collecting duct and further downstream but with intact ENaC function in the connecting tubule have intact urinary acidification. These findings were interpreted as evidence for a major role of the connecting tubule in furosemide-induced urinary acidification (12). de Bruijn et al. showed that benzamil in combination with furosemide only partially blunted urinary acidification, pointing to more than one site of action.

There are several unresolved issues with the new findings. The model by de Bruijn et al. (5) predicts that furosemide should stimulate NHE3-dependent bicarbonate reabsorption by the TAL. However, Capasso et al. (4) showed >20 yr ago that bumetanide but not furosemide stimulated bicarbonate reabsorption by the TAL in micropuncture experiments. In contrast, Good and colleagues (7) showed that furosemide increases bicarbonate absorption in in vitro microperfusion experiments. The difference between these reports might be related to the different techniques and also to the influence osmolality has on TAL bicarbonate reabsorption. High ambient osmolality reduces NHE3-dependent bicarbonate absorption, whereas low osmolality stimulates bicarbonate absorption (6, 13, 20). Of note, most in vitro experiments in the study by de Bruijn et al. were in the absence of bicarbonate and ammonium and with normal osmolality, conditions unlikely occurring in vivo. In the presence of the NHE3 inhibitor, mice failed to acidify their urine after furosemide. However, a residual effect remained, suggesting that NHE3-independent mechanisms may be operative. However, it is not clear whether the NHE3 inhibitor exerted full inhibition as one may expect some degree of bicarbonate losses. Clinically, the $F+F$ test consists of the combined application of a mineralocorticoid and a loop diuretic. Experiments by both Kovacikova et al. (12) and de Bruijn et al. (5) were performed without priming ENaC activity. It would be desirable to repeat these experiments in genetically well-defined mouse models after maximizing ENaC activity to delineate the contribution of the different segments and transport mechanisms to overall urinary acidification.

Addressing some of these issues will certainly provide new insights into the role of the TAL in urinary acidification and the interplay between the TAL and the connecting tubule and collecting duct in this process and may further refine the interpretation of this clinically very useful test. In this respect, it would certainly also be very interesting to learn more from the results of the $F+F$ test in patients with identified molecular defects as to whether mutations in the proton pump subunits show different effects compared with patients with SLC4A1 mutations or patients with RTA of other causes. SLC4A1 is not expressed in the TAL, whereas the proton pump is present.

Taken together, the model of furosemide-induced stimulation of TAL NHE3 activity and subsequent urinary acidification challenges our “textbook knowledge” and sheds new light on the significance of this nephron segment in renal acid-base handling. The findings also suggest that the urinary acidification induced by the $F+F$ test may be composed of at least two additive effects: one located in the TAL and one in the connecting tubule and collecting duct. Their relative contribution to total urinary acidification remains to be determined.

DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS
Author contributions: C.A.W. drafted manuscript; C.A.W., N.M., and S.B. edited and revised manuscript; C.A.W., N.M., and S.B. approved final version of manuscript.

REFERENCES


