Furosemide-induced urinary acidification is caused by pronounced H\(^+\) secretion in the thick ascending limb

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The loop diuretic furosemide is a broad sodium channel inhibitor, which is traditionally attributed to its ability to acutely induce urinary acidification. The current understanding of furosemide-induced urinary acidification is the following: by inhibiting NaCl reabsorption in the TAL, the collecting duct (CD) is exposed to an increased tubular Na\(^+\) load, providing more substrate for electrogenic Na\(^+\) absorption. The epithelial Na\(^+\) channel (ENaC)-mediated reabsorption will result in a lumen-negative transepithelial voltage, which, in turn, is believed to increase H\(^+\) secretion by V-type H\(^+\)-ATPase localized to the apical membrane of α-ICCs. It is well established that amiloride and other ENaC blockers inhibit this urinary acidification by furosemide, which is in agreement with the above explanation (18).

Although still widely accepted, this theory has been challenged by theoretical calculations, which question if increased transtubular voltage in the cortical CD (CCD) will result in the proposed increased acid secretion (27). In addition, studies have shown that mice lacking ENaC in the CD exhibit furosemide-induced urine acidification similar to wild-type mice and that mice lacking the B1 subunit of H\(^+\)/H\(_1\)/Na\(^+\)/Cl\(^-\) (H\(_11001\)) exchanger do not show significant furosemide-induced net acid excretion (NAE) (20) despite the absence of urinary acidification (8).

Previous results have shown that HCO\(_3^-\) reabsorption in the TAL is dependent on Na\(^+\)/H\(^+\) exchanger (NHE) activity and is stimulated by furosemide (12–14). In the present study, we hypothesized that furosemide can also increase H\(^+\) secretion in the medullary TAL (mTAL) via NHEs, independent of HCO\(_3^-\) absorption, which contributes to loop diuretic-induced urinary acidification. First, we tested if furosemide directly affects H\(^+\) secretion in the mouse isolated perfused mTAL. Second, we studied, by pharmacological inhibition, if furosemide-induced tubular acid secretion in the mTAL is specifically mediated by apical Na\(^+\)/H\(^+\) exchange, namely, NHE3. Third, we tested if specific pharmacological blockage of NHE3 in whole animals reduced the furosemide-induced urinary acidification and NAE. Finally, we tested, in vivo, if furosemide was able to cause urinary acidification after blockage of ENaC with benzamil.

Here, we provide evidence that furosemide acutely induces urinary acidification by increasing acid secretion directly in the TAL. We also show that this partially occurs independent of ENaC. These results provide a major addition and revision of the physiological understanding of renal tubular acidification. They may also have important implications for the interpretation of the furosemide test used to diagnose dRTA. These results may lead to a better classification of RTA as well as a more comprehensive understanding of the mechanism underlying acute loop diuretic-induced urinary acidification.

MATERIALS AND METHODS

Animals. Experiments were performed in 4- to 6-wk-old mice of either sex. Animals had free access to standard rodent diet and tap water. Mouse handling and breeding were approved by Danish animal welfare regulations (Dyreforførslighedsny, approval no. 2005/562-31). Mice were of a mixed genetic background (B6D2/SV129) and were bred in house.

Tube perfusion. Mice were euthanized by cervical dislocation. The kidneys were removed, placed, and sliced in ice-cold (4°C) control solution (see below). mTALs were isolated from the inner stripe of the outer medulla with ultrafine forceps. Dissected mTALs were transferred to a tissue chamber and perfused with a concentric pipette system, as previously described (16), with control solution at 37°C. TALs were stabilized on the bath bottom with a holding pipette. The perfusion chamber was mounted on an inverted fluorescence microscope (Axiovert 100 TV, Zeiss, Germany). The setup comprised an inverted microscope with a ×63 C-Apochromat 1.2 numerical aperture water (used for intracellular pH (pHi) and Na⁺ measurements) and ×25 LD Plan-apochromat 0.8 numerical aperture objective (Zeiss) (used for luminal extracellular pH measurements), a VisiChrome polychromator (Visitron), and a digital charge-coupled device camera (Spot Pursuit 1.4 monochrome, Diagnostic Instruments). Images were acquired and data were analyzed with standard software (VisiView, Visitron).

pH measurements. pHi was measured with the ratiometric fluorescent dye BCECF-AM (Invitrogen). Tubules were incubated with 5 μM BCECF-AM in control solution for 20 min at room temperature during continuous luminal perfusion followed by a 5-min basolateral washout period. pHi was measured every 5 s as the emission ratio at 490/436-nm excitation. The excitation duration was 50 ms at 436 nm and 50-ms exposure to excitation light at 488 nm every 10 s (emission filter: 520–560 nm). The entire tubule was used for analysis of the luminal furosemide (1 mg/ml) mix (0.1 ml/10 g body wt) and were held under anesthesia with a ketamine (10 mg/ml)-xylazine (1 mg/ml) mix (0.1 ml/10 g body wt) and were held under anesthesia for 3 h with a third of the original dose every 30 min. A bladder catheter was placed through a small abdominal incision. A micro-pH electrode and a reference electrode with a 100-μm tip (Unisense) were placed directly into the outflow of the catheter, and urine pH was recorded at 5-s intervals. Urine was collected in glass capillaries for further analysis of NAE. A subset of animals received a subcutaneous injection of 4 μg/g body wt #4167 (Aventis) 30 min after urine collection was initiated and received a second dose after another 30 min. A subset of both control animals and animals that received #4167 was injected with 2 μg/g body wt furosemide (Furix, Nycomed) after 60 min.

NAE is the quantification of total H⁺ excretion in the urine. The NAE rate was defined as follows: (NH₄⁺ concentration − HCO₃⁻ concentration + titratable acid concentration) × volume × time⁻¹. HCO₃⁻ concentrations were measured immediately after collection into glass capillaries with an infrared CO₂ sensor (GMT221 CAR-BOCARP, Vaisala, Finland). NH₄⁺ and total phosphate were determined with standard ion-exchange chromatography. The concentration of titratable acid was defined by measuring urinary pH and the concentration of phosphate. Concentrations of H⁺ buffered by phosphate were calculated with standard acid dissociation constants.

Solutions and chemicals. Experiments were performed at 37°C with control solution containing (in mM) 145 NaCl, 3.6 K-gluconate, 1.3 Ca-gluconate, 1 MgCl₂, and 5 D-glucose along with 100 μM BCECF acid titrated to pH 7.4. The lumen was filled with the dye-containing solution, the perfusion was discontinued, and the tubular outflow was closed. pHₐ was measured for 5 min by the same procedure as described above for pHi. The drugs (furosemide and amiloride) were included in the perfusion solution.

Calibration of the BCECF signal was performed using nigericin in the presence of high K⁺ concentration (25) in every experiment. The calibration solution contained (in mM) 95 KCl, 15 NaCl, 0.4 NaH₂PO₄, 1.6 NaHPO₄, 5 glucose, 1 MgCl₂, 1.3 Ca-gluconate, 25 HEPES, and 20 N-methyl-d-glucamine supplemented with 2 μM nigericin. Calibration solutions were titrated to pH 6.5, 7.4, and 7.8 at 37°C.

Intracellular Na⁺ measurements. Changes in intracellular Na⁺ were determined using the nonratiometric Na⁺-sensitive fluorescent dye CoroNa green (Invitrogen). Tubules were incubated with 10 μM basolateral CoroNa green-AM for 30 min at room temperature followed by a 5-min washout. CoroNa fluorescence was measured by 50-ms exposure to excitation light at 488 nm every 10 s (emission filter: 520–560 nm). The entire tubule was used for analysis of the fluorescence signal. Experiments with fluorescence decay over 30% were discarded from further analysis.

Measurement of urinary NAE. Urine collection was performed in mice by bladder catheterization after gavage volume loading (0.6 ml saline). Mice were anesthetized with a ketamine (10 mg/ml)-xylazine (1 mg/ml) mix (0.1 ml/10 g body wt) and were held under anesthesia for 3 h with a third of the original dose every 30 min. A bladder catheter was placed through a small abdominal incision. A micro-pH electrode and a reference electrode with a 100-μm tip (Unisense) were placed directly into the outflow of the catheter, and urine pH was recorded at 5-s intervals. Urine was collected in glass capillaries for further analysis of NAE. A subset of animals received a subcutaneous injection of 4 μg/g body wt #4167 (Aventis) 30 min after urine collection was initiated and received a second dose after another 30 min. A subset of both control animals and animals that received #4167 was injected with 2 μg/g body wt furosemide (Furix, Nycomed) after 60 min.

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Fig. 1. Luminal furosemide triggers a marked intracellular alkalization in the isolated perfused mouse mTAL. A shows an original recording of pHi in a mouse mTAL bathed in HEPES-buffered solution. The addition of luminal furosemide (100 μM) rapidly caused a marked intracellular alkalization, from pH 7.3 to 7.7. The alkalization remained stable throughout the application of furosemide. The
Furosemide effect was reversible, with a mean increase in pH from 7.27 ± 0.06 to 7.60 ± 0.04 (n = 10; Fig. 1B). Moreover, the effect of furosemide was repeatable on the same tubule (data not shown). In a second series, we studied the effect of luminal furosemide in CO2/HCO3− buffer (Fig. 1C). In the presence of CO2/HCO3−, luminal furosemide alkalized the mTAL cytosol from pH 6.95 ± 0.03 to 7.07 ± 0.04 (n = 5; Fig. 1D). Transposition of the furosemide-induced alkalizations to reductions in steady-state intracellular H+ concentrations indicated that under both buffer conditions, virtually the same shift of H+ concentrations was elicited (change in H+ concentration in HEPES: 31.3 ± 8.7 nM and change in H+ concentration in CO2/HCO3−: 32.2 ± 5.5nM). In summary, luminal furosemide triggered a substantial intracellular alkalization in the mouse mTAL.

**Effect of basolateral amiloride on furosemide-induced intracellular alkalization.** The pronounced furosemide-induced intracellular alkalization is likely to result from an increased activity of luminal NHE3. Therefore, we tested the specific NHE3 blocker #4167. Similar to amiloride, #4167 inhibited the furosemide-induced alkalization (1 μM; Fig. 3, C–E). These data indicate that the furosemide-induced alkalization is caused by an increased activity of luminal NHE3.

**Effect of luminal furosemide and amiloride on intraluminal pH.** The above data imply that furosemide stimulates apical NHE3-dependent H+ efflux and causes an intraluminal acidification. To measure this, we measured pHlum in the isolated perfused mTAL. Filling the tubular lumen with BCECF acid and subsequently closing the outflow of the tubule allowed for the recording of the fluorescence signal for over 5 min, resulting in pHlum of 6.92 ± 0.04 (n = 6). Refilling with the same
Washout of furosemide resulted in a recovery of pH_{lum} (Fig. 4A; Fig. 4B shows a summary). These results are consistent with furosemide triggering a luminal acidification that mirrors the intracellular alkalization. Furthermore, we studied the effect of luminal amiloride on pH_{lum}. Amiloride (1 mM) caused a significant alkalization of the tubular fluid (Fig. 4, C and D). These data support that the baseline resting activity of the apical amiloride-sensitive NHE acidifies the lumen, which is stimulated by furosemide.

**Luminal furosemide abruptly reduces cytosolic Na\(^+\) in the mTAL.** Subsequently, we studied how furosemide leads to increased activity of the apical NHE3. Furosemide is known to block apical Na\(^+\) influx (15). The continuously active basolateral Na\(^+\)-K\(^+\)-ATPase could thus potentially reduce cytosolic Na\(^+\) concentration, because the efflux of cytosolic Na\(^+\) would surpass the influx. To measure intracellular Na\(^+\) concentration ([Na\(^+\)]\(_{i}\)) in the mTAL, we used the Na\(^+\)-sensitive fluorescent dye CoroNa green-AM. In all experiments, furosemide caused an abrupt and substantial drop in [Na\(^+\)]\(_{i}\) (Fig. 5, A and C), which was not observed during perfusion with vehicle (Fig. 5, B and C). Prompt lowering of [Na\(^+\)]\(_{i}\) will increase the chemical gradient for Na\(^+\) entry via NHE3 and thus favor H\(^+\) secretion. By this mechanism, we propose that furosemide can directly augment the driving force for H\(^+\) secretion via NHE3.

**Furosemide stimulates NHE3-dependent H\(^+\) excretion in mice.** If the above-described furosemide-induced H\(^+\) secretion from the TAL is of a substantial magnitude, it could explain the furosemide-induced urinary acidification and increased NAE. To study this, we established whole animal experiments, which allowed continuous measurements of urinary pH and simultaneous urine collections for 3 h. Figure 6A shows the urinary pH measurements of control mice and mice that received a bolus injection with furosemide (2 μg/g body wt). Furosemide caused a massive urinary acidification while at the same time increased the urinary output about threefold (Fig. 6B). We then used the specific NHE3 blocker #4167 (23) to test if coappli-

![Fig. 4. Luminal furosemide causes a reversible acidification of the luminal fluid in the mTAL. A and B: original trace of intraluminal pH (pH_{lum}) determined with BCECF acid (A) and summary (B; n = 6, *P < 0.001). C and D: luminal amiloride caused a reversible alkalization of the luminal fluid in the mTAL, as shown in the original trace (C) and summary (D; n = 10, *P < 0.008).](http://ajprenal.physiology.org/)

![Fig. 5. Luminal furosemide abruptly reduced CoroNa green fluorescence as a measure of intracellular Na\(^+\) concentration in the perfused mTAL. A and B: normalized traces of CoroNa green fluorescence intensity in response to furosemide (A; n = 5) and vehicle (B; n = 6). C: summary of the fluorescence drop measured 5 min after perfusion of either furosemide (100 μM) or vehicle (*P < 0.0006).](http://ajprenal.physiology.org/)
cation with furosemide could affect the amount of furosemide-induced NAE. From our single tubule experiments, one would expect that #4167 alkalizes the urine. Therefore, we tested how much #4167 was necessary to provoke a sizeable increase in urinary pH. A dose of 4 μg/g body wt increased urinary pH from 6.91 ± 0.18 to 7.37 ± 0.22, and urinary HCO₃⁻ concentrations were 1.14 ± 0.32 mM before and 5.33 ± 3.63 mM after drug application (n = 8, P = 0.25).

Moreover, we tested the specificity of #4167 as a NHE3 blocker to account for possible interference with ENaC. This was done in a preparation of the murine distal colon in a Ussing chamber. As shown in Fig. 7, #4167 had no effect on ENaC up to a tested concentration of 100 μM, whereas amiloride robustly inhibited electrogenic Na⁺ transport. These experiments support that #4167 specifically inhibits NHE3 and that it is possible to induce a sizeable inhibition in vivo in the renal tubule.

As shown in Fig. 6C, inset, application of 4 μg/g body wt #4167 stably reduced urinary H⁺ concentration (alkalization, green curve). Application of furosemide (red curve) led to a marked increase of H⁺ concentration. In experiments with #4167 and furosemide (blue curve), an increase of H⁺ concentration was absent during the first 15 min and only after this time was a slow and small increase of H⁺ concentration seen. The peak H⁺ concentration of 14.89 ± 9.86 μM in the furosemide-alone series was reached after 15 min compared with the peak H⁺ concentration of 2.93 ± 1.15 μM reached after 49 min in the #4167 and furosemide series. These data indicate that inhibition of the NHE3 antiporter substantially reduced the furosemide-induced H⁺ excretion. To further quantify this effect of NHE3 blockade on furosemide-stimulated acid excretion, NAE was measured 30 min before and after the application of furosemide. Figure 8 shows NAE excretion rates for all four experimental series. All relevant data that form the basis for NAE calculations are shown in Fig. 9. Precontrol NAE rates were similar in all experimental series (Fig. 8). The addition of furosemide alone markedly increased NAE (3-fold). This increase amounted to 6.52 ± 2.02 μmol/h (n = 8) in the series where furosemide was applied alone (Fig. 8B). In the series where furosemide was given with #4167, NAE also increased significantly after the addition of furosemide but was markedly reduced to <30% (1.87 ± 0.66 μmol/h). These data indicate that blockade of NHE3 substantially diminishes the ability of furosemide to increase NAE in the mouse.

Furosemide-stimulated urinary acidification is partially preserved during ENaC blockage. The above data propose that the furosemide-induced urinary acidification occurs independent of ENaC. To test this more directly, we performed in vivo experiments and applied furosemide after ENaC inhibition with 2 μg/g body wt benzamil (10, 20). ENaC blockage with benzamil or amiloride elicited a fast and stable urinary alkalization (benzamil: from 6.64 ± 0.11 to 7.34 ± 0.16, n = 4; amiloride: from 6.54 ± 0.17 to 7.04 ± 0.06, n = 4; Fig. 10A). The addition of furosemide in the continuous presence of benzamil caused a marked and fast urinary acidification down to pH 5.79 ± 0.18 (n = 4). The increased H⁺ excretion by furosemide was attenuated during ENaC inhibition (Fig. 10B). These results support that a significant part of the furosemide-induced urinary acidification occurs independent of functional ENaC. These data also confirm that ENaC function is partially required for furosemide-induced urine acidification. In summary, this strengthens the main finding of this study that furosemide activates NHE3-mediated H⁺ secretion in the TAL.

DISCUSSION

The mechanism by which loop diuretics alter renal water and salt handling have been clearly documented (15). In contrast, the mechanism of their effect on urinary acidification is less well established. In numerous studies, furosemide has been...
shown to induce urinary acidification in various species, including humans (4, 18, 26). Several mechanisms have been suggested to explain the acid excretion induced by loop diuretics. The widespread theory assumes that inhibition of Na\(^+/\)H\(^+\) reabsorption in the TAL triggers effects in the connecting tubule (CNT) and CD. The reduced Na\(^+\) transport in the TAL increases the distal tubular Na\(^+\)/H\(^+\) load and augments ENaC-dependent Na\(^+\)/H\(^+\) absorption. This consequently increases the lumen-negative transepithelial voltage in the CNT and CD, which is believed to increase V-type H\(^+\)-ATPase activity in α-ICs by increasing the driving force for H\(^+\) secretion. This concept has been favored in many studies and reviews (1, 2, 11, 20). A second theory proposes a local action of the diuretic, in which the Na\(^+\)/H\(^+\)-K\(^+\)/H\(^+\)-2Cl\(^-\) cotransporter colocalizes with apical NHEs (12). By inhibiting Na\(^+\)/H\(^+\) influx with loop diuretics, [Na\(^+\)/H\(^+\)]\(_i\) could fall, to an extent that would favor H\(^+\) efflux via NHEs (27). In principle, both mechanisms also could work in parallel to account for the pronounced urine acidification induced by loop diuretics. The present study supports that apical NHE3 in the TAL contributes substantially to furosemide-induced urinary H\(^+\) secretion.

The concept that increased distal Na\(^+\) delivery causes tubular acidification via voltage-driven H\(^+\) secretion was originally developed in the turtle bladder (19). Translation of the voltage-dependent H\(^+\) secretion into the perfused rabbit CCDs, however, rendered controversial results (21, 22). In both of these studies, H\(^+\) secretion was measured as net HCO\(_3\)\(^-\) absorption. Ouabain, which completely abolished the lumen-negative voltage in both studies, only altered H\(^+\) secretion in one of the studies (21). Thus, the theory that lumen-negative transepithelial voltage drives H\(^+\) secretion via apical H\(^+\)-ATPase in native CDs remains unresolved.

A study with CD-specific knockout of ENaC showed that furosemide-induced urinary acidification and increased NAE prevailed in these mice. This was interpreted to highlight the
connect the tubule as the most important ENaC-expressing segment and the site of furosemide-induced urinary acidification (20). This study also reported on the effect of furosemide in Atp6v1b1 knockout mice, which lack functional H⁺ secretion via V-type H⁺-ATPase, and found a substantial furosemide-induced increase in NAE in these animals compared with wild-type controls. Although the CNT may still be a possible site of furosemide-triggered urinary acidification, these data may also imply that this urinary acidification takes place in the TAL. The present study provides evidence for furosemide-induced urinary H⁺ secretion directly via apical NHE3 in the mTAL. Furosemide triggers a massive intracellular alkalization, which results from activation of NHE3-mediated H⁺ secretion. NHE3 activation may be explained directly by a marked reduction of [Na⁺], by luminal furosemide, although more refined signaling pathways could potentially be involved. In whole animal experiments, we provide evidence that specific NHE3 inhibition causes a substantial fall in furosemide-induced NAE.

NHE3 function is important for HCO₃⁻ absorption in both the proximal tubule and TAL. Inhibition of NHE3 will therefore cause urinary HCO₃⁻ wasting, which potentially could originate from either site. We titrated #4167 to a dosage that slightly but convincingly increased urinary pH but avoided massive urinary HCO₃⁻ loss. We observed a tentative increase of urinary HCO₃⁻ with the NHE3 blocker consistent with NHE-dependent inhibition of HCO₃⁻ absorption. We also observed that a dosage of 10 μg/g body wt caused a massive urinary alkalization, which resulted in fast fatal outcome. We assume that #4167 will be upconcentrated in the preurine similar to furosemide, resulting in a higher concentration of #4167 in the TAL compared with the proximal tubule. By choosing a threshold concentration of #4167, which only marginally increased the urinary HCO₃⁻ concentration, we assume to preferentially target NHE3 in the TAL.

Amiloride is a potent blocker of ENaC and therefore abolishes lumen-negative voltage in the CNT/CD (4, 20). The effectiveness of amiloride and other ENaC blockers to inhibit furosemide-induced urinary acidification in animal models and humans has been a key argument for the voltage-mediated H⁺ secretion hypothesis and is therefore conflicting with our data (4, 18, 20). In the present study, we reinvestigated the role of ENaC inhibition in furosemide-induced urinary acidification. We observed a pronounced urinary alkalization by both amiloride and benzamil, of which the mechanism is at present not understood. The urinary acidification by furosemide after ENaC inhibition was still observed, albeit smaller than with active ENaC (see Figs. 6 and 10). These results indicate that the furosemide-induced urinary acidification is partially preserved during ENaC inhibition and thus is not fully dependent on electrogenic Na⁺ absorption. This contrasts the prevailing concept that furosemide-induced urinary acidification fully requires functional ENaC (1, 2, 11, 20). One explanation for this discrepancy may be derived from closer inspection of the red curve shown in Fig. 10A. As mentioned, pretreatment with benzamil causes a marked alkalization, which is followed by furosemide-induced acidification. In previous studies, the urine was collected in summer periods to cover both the alkalizing and acidifying components and may potentially have caused an underestimation of the furosemide-induced acidification. In our experiments, the very high time resolution of the urinary pH measurements unmasked the opposing effect of ENaC blockers and furosemide.

Knockout of the B1 subunit of vacuolar H⁺ ATPase, which is specifically localized to ICs, leads to a marked urinary alkalization, indicating its key importance for H⁺ secretion into the urine (8). Importantly, B1 knockout mice show a greatly blunted urine pH effect after furosemide but an increase of NAE is still present, albeit reduced (20). It has been suggested that vacuolar H⁺-ATPase is required for furosemide-induced urine acidification. The prevailing increase of NAE in the above study suggests the existence of additional mechanisms. Notably, the B1 knockout mouse has a very complex renal salt and water wasting phenotype, which may also include an altered function of renal NHE3 (17). The complex phenotype of the B1 knockout mouse potentially precludes the interpretation that furosemide-induced urine acidification depends solely on vacuolar H⁺-ATPase.

In the presence of #4167, furosemide triggered a greatly inhibited and delayed urinary H⁺ secretion consistent with a NHE3-dependent mechanism. However, the furosemide-induced urinary acidification was not completely blocked by #4167. The intratubular concentrations of #4167 may not have reached 1 μM, which is the concentration required to fully block furosemide-induced alkalization in the isolated tubule. If, however, NHE3 is fully blocked at the given dose, an additional way for furosemide to inflict the residual urinary acidification must be present, namely, increased H⁺ secretion in more distal parts of the tubule. The voltage-influenced model of H⁺ secretion could be responsible for the remaining urine acidification. In addition, furosemide is well established to increase kaliuresis, and increased K⁺ delivery to the CD could cause an increase of H⁺ secretion via distal tubular H⁺-K⁺-ATPases (6).

Several elegant studies are in strong support of the TAL as an important site for furosemide-induced H⁺ secretion. First, data from David Good’s laboratory (5, 13) have shown that the TAL absorbs ~15% of tubular HCO₃⁻ via tubular H⁺ secretion predominantly mediated by apical NHEs. The finding that loop diuretics increase HCO₃⁻ absorption in the TAL (5, 12) is fully consistent with furosemide-mediated activation of NHE3. Second, luminal furosemide has been shown to alkalize rabbit macula densa cells, which are functionally similar to TAL cells. This was fully inhibited by luminal amiloride (9). Finally, pH measurements by micropuncture in the rat early distal convoluted tubule showed that furosemide inflicted a drop in luminal pH (18). Importantly, the early distal tubule is proximal to the site of ENaC expression, excluding ENaC-mediated voltage effects to play a role in this observation.

Taken together, these results, in combination with the available literature, confirm that the TAL is an important site of tubular H⁺ secretion. Moreover, urinary acidification via apical NHE3 is markedly stimulated by luminal furosemide. The present study describes the mechanism of furosemide-induced H⁺ secretion in the TAL and demonstrates that the TAL is an essential site for loop diuretic-induced urinary acidification. This study also shows that furosemide-induced urinary acidification is partially preserved after blockade of ENaC. If these mouse data are similar in humans, these results may have clinical implications in the diagnosis of patients with a reduced ability to excrete urinary acid (RTA). The diagnostic furosemide test should be critically reevaluated to also encompass
the possibility of a dysfunctional TAL as an underlying component of reduced urinary acidification.

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DISCLOSURES

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

AUTHOR CONTRIBUTIONS


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