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Apical and Basolateral Na^+/H^+ Exchange in the Rabbit Outer Medullary Thin Descending Limb of Henle: Role in Intracellular pH Regulation

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Summary. The present study was designed to investigate the apical and basolateral transport processes responsible for intracellular pH regulation in the thin descending limb of Henle. Rabbit thin descending limbs of long-loop nephrons were perfused in vitro and intracellular pH (pH_i) was measured using BCECF. Steady-state pH_i in HEPES buffered solutions (pH 7.4) was 7.18 ± 0.03. Following the removal of luminal Na^+, pH_i decreased at a rate of 1.96 ± 0.37 pH/min. In the presence of luminal amiloride (1 mM), the rate of decrease of pH_i was significantly less, 0.73 ± 0.18 pH/min. Steady-state pH_i decreased 0.18 pH units following the addition of amiloride (1 mM) to the lumen (Na^+ 140 mM lumen and bath). When Na^+ was removed from the basolateral side of the tubule, pH_i decreased at a rate of 0.49 ± 0.05 pH/min. The rate of decrease of pH_i was significantly less in the presence of 1 mM basolateral amiloride, 0.29 ± 0.04 pH/min. Addition of 1 mM amiloride to the basolateral side (Na^+ 140 mM lumen and bath) caused steady-state pH_i to decrease significantly by 0.06 pH units. When pH_i was acutely decreased to 5.87 ± 0.02 following NH_4Cl removal (lumen, bath), pH_i failed to recover in the absence of Na^+ (lumen, bath). Addition of 140 mM Na^+ to the lumen caused pH_i to recover at a rate of 2.17 ± 0.59 pH/min. The rate of pH_i recovery was inhibited 93% by 1 mM luminal amiloride. When 140 mM Na^+ was added to the basolateral side, pH_i recovered only partially at 0.38 ± 0.07 pH/min. Addition of 1 mM basolateral amiloride inhibited the recovery of pH_i by 97%. The results demonstrate that the rabbit thin descending limb of long-loop nephrons possesses apical and basolateral Na^+/N^+ antiporters. In the steady state, the rate of Na^+-dependent H^- flux across the apical antiporter exceeds the rate of Na^+-dependent H^+ flux via the basolateral antiporter. Recovery of pH_i following acute intracellular acidification is Na^+ dependent and mediated primarily by the luminal antiporter.

Key Words Na^+/H^+ exchange · thin descending limb of Henle · BCECF · intracellular pH · pH regulation · fluorescence

Introduction

Previous studies from this laboratory have demonstrated that the rabbit S_3 proximal tubule, in contrast to the more proximal S_2 segment, possesses a Na^+-independent plasma membrane H^+-ATPase which plays an important role in intracellular pH regulation following acute acid loading (Kurtz, 1987). Whether in the outer medulla the S_3 H^+-ATPase activity extends distally into the outer medullary thin descending limb of Henle (tDL) was not previously investigated. The tDL (as all nephron segments), has a requirement to regulate pH_i during systemic acid base disorders to prevent marked changes in cell function and metabolism. However, there are no studies of pH regulation in this segment. Previous studies have failed to provide evidence for net transepithelial transport of Na^+ in the rabbit outer medullary tDL perfused and bathed in identical solutions (Kokko, 1970; Imai, 1984). From these findings it might be assumed that the apical and basolateral membranes of the outer medullary tDL do not possess Na^+-dependent transport pathways and that the pH_i regulatory processes are Na^+-independent. Since no information is available addressing this issue, the present study was designed to determine the apical and basolateral membrane transport pathways responsible for pH_i regulation in the rabbit outer medullary tDL.

Materials and Methods

Tubule Perfusion

Male New Zealand white rabbits weighing approximately 2 kg were sacrificed by cervical dislocation. The right kidney was removed and cut into coronal slices. tDL's of long-loop nephrons were dissected from the inner stripe of the outer medulla with the distal 0.12 ± 0.01 mm of the S_3 proximal tubule left attached to the proximal end of the thin descending limb (Fig. 1). The mean length of the dissected tDL was 1.07 ± 0.03 mm. The tubule was then transferred to the stage of a previously described microfluorometer coupled to the perfusion apparatus (Kurtz, 1987). Tubules were mounted on concentric glass pipets as previously described and perfused by cannulating the S_3 proximal tubule and advancing the perfusion pipet into the lumen of the tDL. The bathing solution could be exchanged with a different solution in <2 sec. The perfusate could be completely changed in <5 sec.

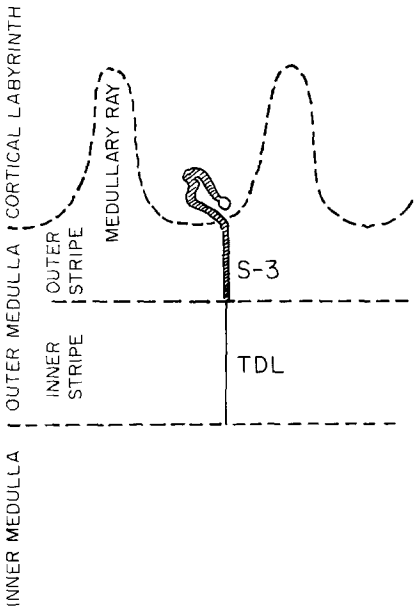

Fig. 1. Anatomical localization of the thin descending limb of long-loop (deep) nephrons

Fluorescent Measurement of pH_i

Intracellular pH (pH_i) was measured using BCECF as previously described (Kurtz, 1987). pH_i was calibrated (from pH 5.8 to 7.3) at the end of each experiment using the high K^+ nigericin technique (Thomas et al., 1979; Kurtz, 1987). Data was acquired from the proximal 0.7 mm of the thin descending limb. The S_3 proximal tubule was not in the field of view when fluorescent measurements were acquired from the tDL.

The initial rate of change of pH_i, dpH_i/dt, was measured in the first 16 sec following a solution change.

Solutions

The composition of the perfusate and bathing solutions was as follows: Na^+, 140 mM; K^+, 5 mM; Ca^+, 1 mM; Mg^+, 1 mM; Cl^-, 144 mM; phosphate, 2.5 mM; glucose, 5 mM; alanine, 5 mM; and HEPES, 5 mM, equilibrated with 100% O_2, pH 7.4. NaCl was replaced with tetramethylammonium chloride when Na^+-free solutions were used. In experiments designed to acutely lower pH_i using the NH_4Cl prepulse technique (Roos & Boron, 1981), 20 mM NH_4Cl replaced 20 mM tetramethylammonium chloride.

Materials

BCECF-AM was from Molecular Probes Inc., Junction City, OR; amiloride hydrochloride dihydrate was from Merck Sharp and Dohme, West Point, PA; tetramethylammonium chloride was from Aldrich Chemical (Milwaukee, WI); nigericin was from Sigma Chemical Co. (St. Louis, MO). None of the compounds used in the present study altered the spectral properties or calibration of BCECF.

Statistics

Results are reported as mean ± SEM. Unpaired Student's t test was used to compare group means.

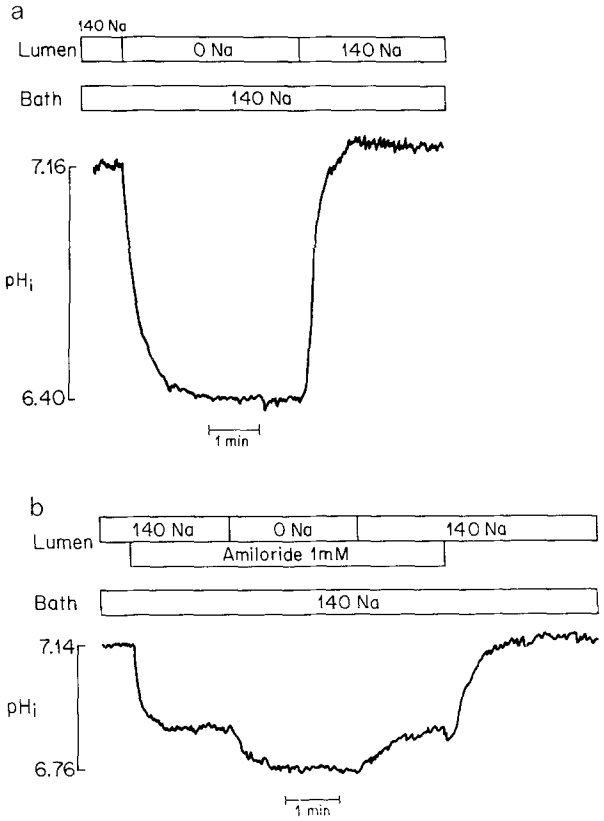

Fig. 2. (*a*) Effect of luminal Na^+ removal and readdition on pH_i in the outer medullary tDL of long-loop nephrons. The tubule was perfused and bathed in HEPES buffered solutions, pH 7.4, in the presence of 140 mM Na^+. Following the removal of luminal Na^+, pH_i decreased at a rate of 1.96 ± 0.37 pH/min (n = 6). Increasing luminal Na^+ to 140 mM resulted in the recovery of pH_i. (*b*) In the presence of amiloride (1 mM, lumen), the rate of decrease of pH_i upon luminal Na^+ removal was significantly less, 0.73 ± 0.18 pH/min, (n = 7), $P < 0.001$

Results

Luminal Na^+ Removal and Addition

Steady-state pH_i in the presence of 140 mM Na^+ (lumen and bath) was 7.18 ± 0.03 (n=22). When luminal Na^+ was decreased from 140 mM to 0, pH_i decreased by 0.61 ± 0.05 pH units, n=6, $P < 0.001$. The initial rate of decrease of pH_i following luminal Na^+ removal was 1.96 ± 0.37 pH/min, n=6, (Fig. 2*a*, Table 1). When 140 mM Na^+ was added to the lumen, pH_i recovered (Fig. 2*a*). To determine whether the Na^+-dependent changes in pH_i were due to H^+ flux on a luminal Na^+/H^+ antiporter, similar studies were performed in the presence of luminal amiloride (1 mM). As demonstrated in Fig. 2*b* and Table 1, amiloride significantly decreased the rate of decrease of pH_i to 0.73 ± 0.18 pH/min, n=7, $P < 0.001$. These results suggest that this nephron

Table 1. Initial rate of decrease of pH_i following Na^+ removal

	Lumen		Bath	
	Control	Amiloride (1 mM)	Control	Amiloride (1 mM)
dpH_i/dt (pH/min)	1.96 ± 0.37	0.73 ± 0.18[a]	0.49 ± 0.04	0.29 ± 0.04[b]
n	6	7	9	5

[a] $P < 0.001$ *vs.* control (lumen).
[b] $P < 0.02$ *vs.* control (bath).

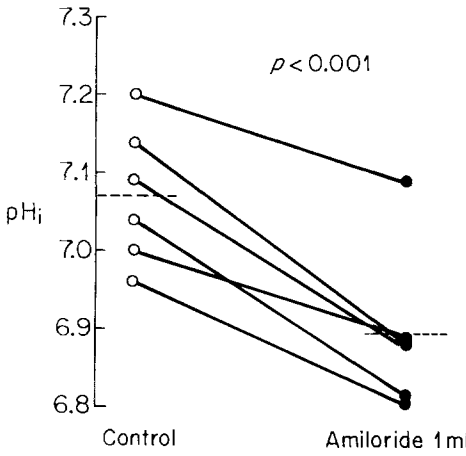

Fig. 3. Effect of the addition of luminal amiloride (1 mM) on pH_i. Amiloride (1 mM) when added to the lumen (Na^+ 140 mM lumen and bath) caused steady-state pH_i to decrease from 7.07 ± 0.04 to 6.89 ± 0.04, $n = 6$, $P < 0.001$ (*see* Fig. 2*b*). The change in pH_i resulting from luminal amiloride addition was reversible (*see* Fig. 2*b*)

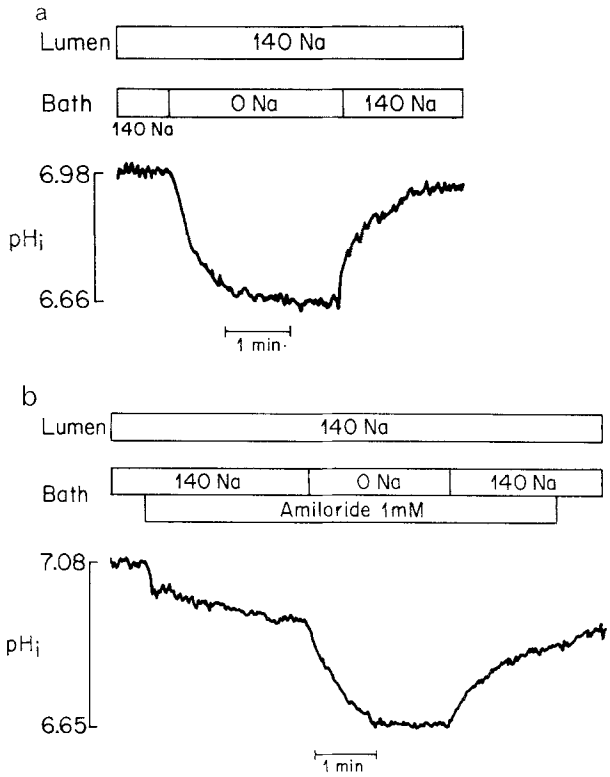

Fig. 4 (*a*) Effect of basolateral Na^+ removal and readdition on pH_i. When basolateral Na^+ was removed, pH_i decreased at a rate of 0.49 ± 0.05 pH/min, $n = 9$. (*b*) In the presence of amiloride (1 mM, bath), the rate of decrease of pH_i following basolateral Na^+ removal was 0.29 ± 0.04, $n = 5$, $P < 0.02$

segment possesses an apical Na^+/H^+ antiporter. Further studies were performed to confirm that the apical antiporter functions under steady-state conditions. In the presence of luminal and basolateral Na^+ (140 mM), the addition of amiloride (1 mM) to the lumen decreased steady-state pH_i from 7.07 ± 0.04 to 6.89 ± 0.04, $n=6$, $P < 0.001$ (Figs. 2*b* and 3), indicating that the antiporter is mediating cellular H^+ efflux into the luminal fluid under steady-state conditions. The change in pH_i induced by luminal amiloride addition was reversible in the experiment depicted in Fig. 2*b*. In other experiments (*not shown*), the amiloride effect was not completely reversible likely due to the incomplete removal of amiloride from its binding site.

Basolateral Na^+ Removal and Addition

Additional experiments were performed to determine whether this segment possesses a basolateral Na^+ coupled H^+ transport pathway. Following basolateral Na^+ removal, pH_i decreased 0.39 ± 0.04 pH units, $n=6$, $P < 0.001$ at a rate of 0.49 ± 0.05 pH/min, $n=9$, (Fig. 4*a*, Table 1). In the presence of basolateral amiloride (1 mM), the rate of decrease of pH_i upon bath Na^+ removal was significantly less than control, 0.29 ± 0.04 pH/min, $n=5$, $P < 0.02$ (Fig. 4*b*, Table 1). These results indicate that the basolateral membrane of the long-loop tDL possesses a Na^+/H^+ antiporter. The magnitude of the amiloride inhibitable initial rate of decrease in pH_i following bath Na^+ removal, 0.20 pH/min, was less than following luminal Na^+ removal, 1.23 pH/min, indicating that under identical initial conditions, the rate of H^+ flux across the apical antiporter exceeds the H^+ flux across the basolateral antiporter. In order to determine whether the basolateral antiporter is functioning under steady-state conditions to mediate cellular H^+ efflux, amiloride (1 mM) was added to the basolateral side of the tubule. In the presence of 140 mM Na^+ (lumen, bath), steady-state pH_i decreased significantly from 7.21 ± 0.04 to 7.15 ± 0.05, $n=8$, $P < 0.01$ (Figs. 4*b* and 5). This latter result indicates that the basolateral antiporter (as the luminal antiporter) functions in the steady

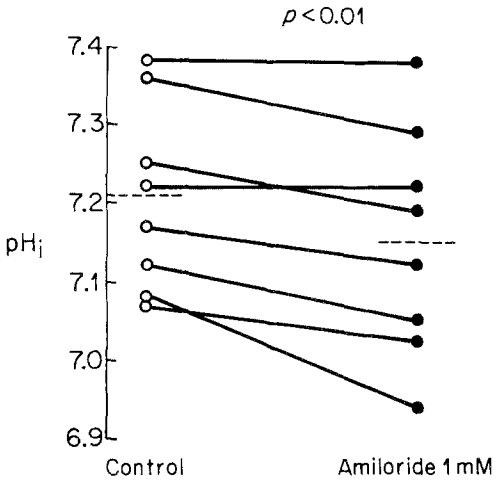

Fig. 5. Effect of the addition of basolateral amiloride (1 mM) on pH_i. The addition of amiloride (1 mM) to the bath caused (Na^+ 140 mM lumen and bath) steady-state pH_i to decrease from 7.21 ± 0.04 to 7.15 ± 0.05, $n = 8$, $P < 0.01$ (*see* Fig. 4*b*)

state. The finding that the addition of amiloride to the lumen causes a greater fall in pH_i than that following the basolateral addition of amiloride is additional evidence that H^+ flux across the apical antiporter exceeds basolateral antiporter-mediated H^+ flux.

Intracellular pH Regulation

The following series of experiments were performed to determine (i) the role of the apical and basolateral Na^+/H^+ antiporters in pH_i regulation following acute intracellular acid loading, and (ii) whether this segment possesses a plasma membrane H^+-ATPase that regulates pH_i. The tubule was exposed to a solution containing 20 mM NH_4Cl for 10 min (lumen and bath). Following the removal of NH_4Cl, pH_i decreased acutely to 5.87 ± 0.02 (n=14) and failed to recover in the absence of luminal and basolateral Na^+ (Figs. 6–9, Table 2). This result suggests that the outer medullary tDL (long-loop segment) lacks a Na^+-independent plasma membrane H^+-ATPase, which contributes importantly to the regulation of pH_i following acute intracellular acidification. Upon the addition of 140 mM Na^+ to the lumen, pH_i recovered from a minimum value of 5.94 ± 0.02 at a rate of 2.17 ± 0.59 pH/min, n=5, $P < 0.001$ (Fig. 6, Table 2). In approximately half the experiments, pH_i increased to a higher value than the pH_i measured in the presence of NH_4Cl. Whenever this "overshoot" was observed, pH_i always decreased subsequently to a lower final value. Figure 6 is an experiment demonstrating the overshoot and subsequent recovery of pH_i following the addition of 140 mM Na^+ to the lumen. As demonstrated in Fig. 7 and Table 2, 1 mM amiloride decreased the initial rate of Na^+-dependent pH_i recovery to 0.16 ± 0.05 pH/min, n=5, $P < 0.01$. Removal of luminal amiloride resulted in an increase in the pH_i recovery rate. The rate of recovery of pH_i following luminal amiloride removal was variable, and in the experiment depicted in Fig. 7., less than the control recovery rate likely because of incomplete removal of amiloride from the tDL apical membrane. These experiments demonstrate that the Na^+-dependent recovery of pH_i is due to a luminal Na^+/H^+ antiporter.

In separate experiments, pH_i was again acutely decreased by NH_4Cl removal in the absence of luminal and basolateral Na^+. Following the addition of 140 mM Na^+ to the basolateral side of the tubule,

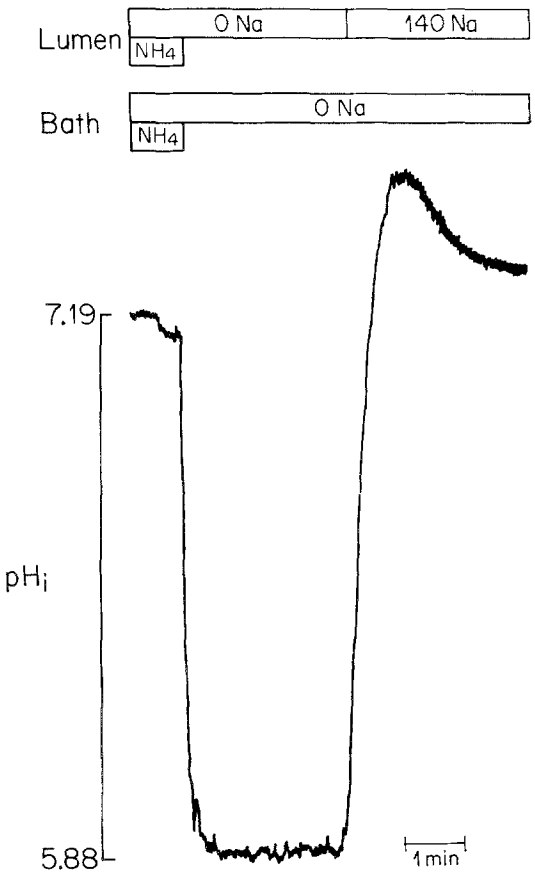

Fig. 6. Recovery of pH_i following acute intracellular acidification. The tubule was exposed to a solution containing 20 mM NH_4Cl (lumen, bath) for 10 min. Following the removal of NH_4Cl, pH_i decreased to 5.87 ± 0.02 (n = 14) as a result of the rapid cellular efflux of NH_3. In the absence of Na^+ (lumen, bath), pH_i failed to recover. When Na^+ (140 mM) was added to the lumen, pH_i recovered at a rate of 2.17 ± 0.59 pH/min. Occasionally, an overshoot of pH_i was observed followed by a subsequent recovery

Table 2. pH_i recovery following acute intracellular acidification by NH_4Cl prepulse

	Na^+ free (lumen, bath)	Na^+ 140 mM (lumen)	Na^+ 140 mM amiloride 1 mM (lumen)	Na^+ (140 mM) (bath)	Na^+ (140 mM) amiloride (1 mM) (bath)
dpH_i/dt (pH/min)	0.02 ± 0.003	2.17 ± 0.59[a]	0.16 ± 0.05[b]	0.38 ± 0.07[a]	0.01 ± 0.004[c]
Minimum pH_i	5.87 ± 0.02	5.94 ± 0.02	5.93 ± 0.06	5.82 ± 0.02	5.84 ± 0.06
n	14	5	5	6	4

[a] $P < 0.001$ *vs.* Na^+ free (lumen, bath).
[b] $P < 0.01$ *vs.* Na^+ 140 mM, lumen.
[c] $P < 0.01$ *vs.* Na^+ 140 mM, bath.

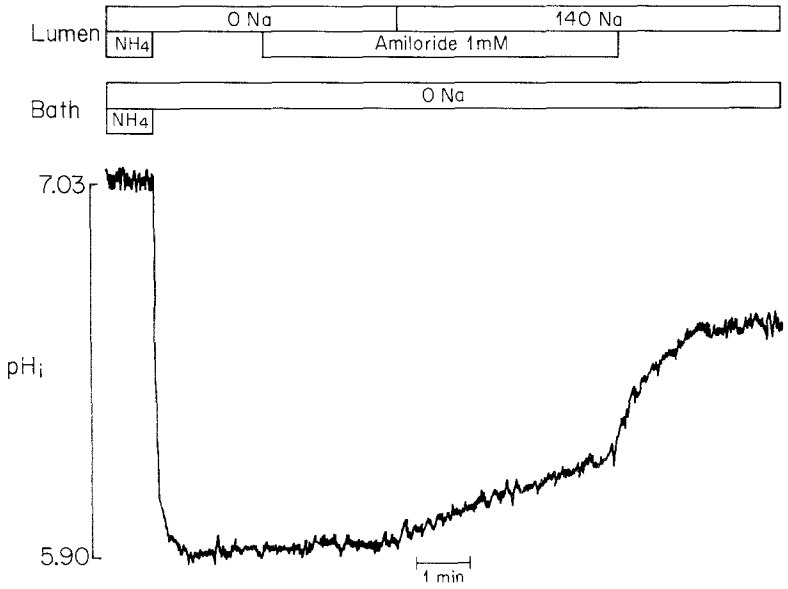

Fig. 7. Recovery of pH_i following acute intracellular acidification by NH_4Cl removal in the absence of Na^+ (lumen, bath): Effect of luminal amiloride. Amiloride (1 mM, lumen) significantly decreased the recovery of pH_i following the addition of Na^+ (140 mM) to the lumen, 0.16 ± 0.05 pH/min, $n = 5$, $P < 0.01$. When amiloride was removed, pH_i increased more rapidly. The pH_i recovery rate following amiloride removal was less than the control recovery rate likely because of incomplete removal of amiloride from its binding site

pH_i recovered partially and increased from a minimum value of 5.82 ± 0.02 at a rate of 0.38 ± 0.07 pH/min, $n=6$, $P < 0.001$ (Fig. 8*a* and *b*, Table 2). When basolateral Na^+ was removed, pH_i decreased (Fig. 8*a*). Following the addition of Na^+ (140 mM) to the lumen, pH_i recovered completely (Fig. 8*a*). In a separate experiment, following the partial recovery of pH_i induced by basolateral Na^+ addition, luminal Na^+ addition resulted in the complete recovery of pH_i (Fig. 8*b*). Addition of 1 mM basolateral amiloride significantly decreased the Na^+-dependent rate of recovery of pH_i to 0.01 ± 0.004 pH/min, $n=4$, $P < 0.01$ (Fig. 9, Table 2). Following the removal of basolateral amiloride, the rate of pH_i recovery increased (Fig. 9), but not always at the control rate, likely because of the incomplete removal of amiloride from the basolateral membrane.

When pH_i had decreased to a minimum value following the NH_4Cl prepulse in the absence of functioning apical and basolateral Na^+/H^+ antiporters (Na^+ absent from lumen and bath), the addition of amiloride (1 mM) to the lumen or bath failed to decreased pH_i further (Figs. 7 and 9).

The results demonstrate that the magnitude and rate of the pH_i recovery following luminal Na^+ addition was significantly greater than following the basolateral addition of Na^+. Of note, pH_i recovered completely following the addition of 140 mM Na^+ to the lumen, but failed to do so following the addition of 140 mM Na^+ to the basolateral side. pH_i failed to recover completely following basolateral Na^+ addition, likely because as the intracellular Na^+ concentration increased as a result of basolateral Na^+ influx, Na^+ began to enter the lumen via the apical Na^+/H^+ antiporter (functioning in the reverse direction), which resulted in cellular H^+ influx. When the rate of H^+ influx via the luminal Na^+/H^+ antiporter was equal to the rate of H^+ efflux via the basolateral antiporter, pH_i ceased to increase. The difference between the magnitude and rate of pH_i recovery

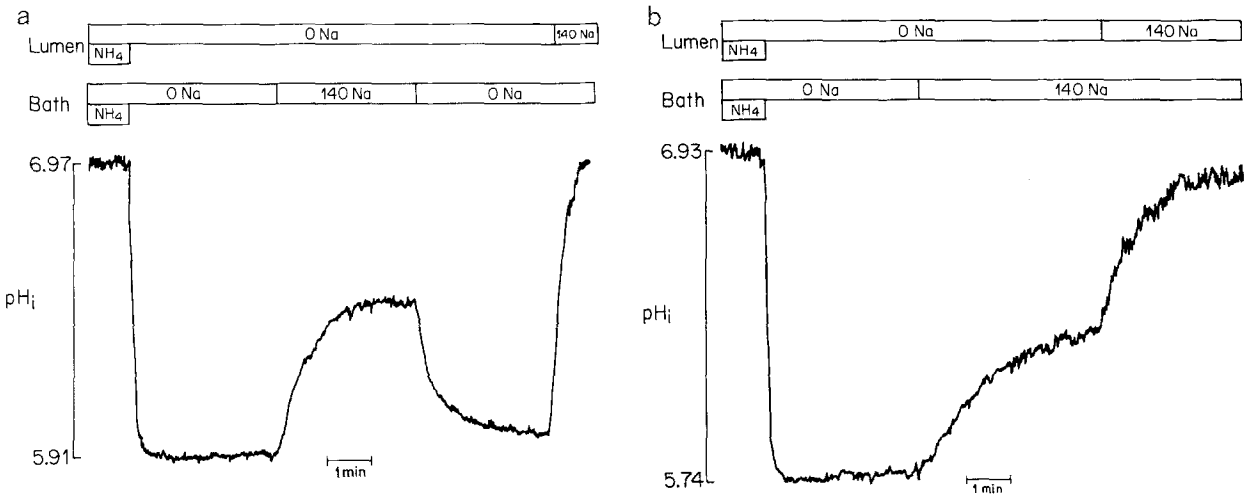

Fig. 8. Recovery of pH_i following acute intracellular acidification by NH_4Cl removal in the absence of Na^+ (lumen, bath): basolateral Na^+ addition. (*a*) Following the addition of Na^+ (140 mM) to the basolateral side of the tubule, pH_i recovered partially at a rate of 0.38 ± 0.07 pH/min, $n = 6$. In order to compare the initial rate of pH_i recovery in the same tubule following luminal Na^+ addition, Na^+ was removed from the basolateral side and pH_i decreased approximately to the same initial value. Addition of Na^+ (140 mM) to the lumen resulted in a more rapid recovery of pH_i. The recovery of pH_i was incomplete following basolateral Na^+ addition, in contrast to the complete recovery of pH_i induced by luminal Na^+ addition. (*b*) In a separate experiment following the partial recovery of pH_i induced by basolateral Na^+ addition, 140 mM Na^+ was added to the lumen, which caused pH_i to recover completely. Note that in this experiment, although the initial value of pH_i was higher when Na^+ was added to the lumen, the rate of pH_i recovery exceeded the recovery rate following basolateral Na^+ addition

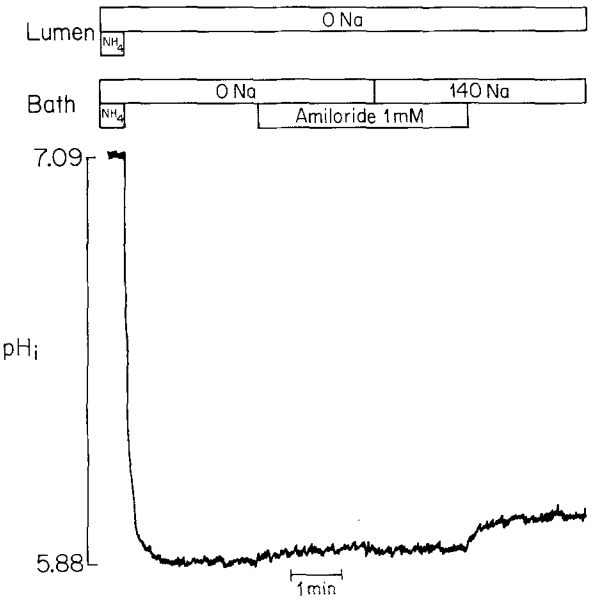

Fig. 9. Recovery of pH_i following acute intracellular acidification by NH_4Cl removal in the absence of Na^+ (lumen, bath): Effect of basolateral amiloride. When 140 mM Na^+ was added to the basolateral side of the tubule in the presence of basolateral amiloride (1 mM), the pH_i recovery rate was significantly decreased to 0.01 ± 0.004 pH/min, $n = 4$, $P < 0.01$. Removal of basolateral amiloride increased the rate of recovery of pH_i. The pH_i recovery rate was less than the control recovery rate likely because of the incomplete removal of amiloride from its binding site

following luminal *versus* basolateral Na^+ addition indicates that the luminal Na^+/H^+ antiporter is quantitatively more important than the basolateral antiporter in regulating pH_i in this tubule segment.

Kinetics

Tubules were perfused in the absence of Na^+ (lumen, bath) and pH_i was acutely acidified by NH_4Cl removal as described earlier. The kinetics of the apical Na^+/H^+ antiporter were determined by measuring the rate of recovery of pH_i following the addition of Na^+ (0–140 mM) to the lumen. The Michaelis constant (K_m) of the apical of Na^+/H^+ antiporter for external Na^+ was 41 ± 7 mM with a maximum velocity (V_{max}) of 2.7 ± 0.2 pH/min. In separate experiments, the kinetics of the basolateral Na^+/H^+ antiporter were determined by measuring the rate of recovery of pH_i following the addition of Na^+ (0–140 mM) to the basolateral side of the tubule. The K_m of the basolateral Na^+/H^+ antiporter for external Na^+ was 72 ± 7 mM with a V_{max} of 0.69 ± 0.04 pH/min. Given that the cell buffer capacity in these studies measured by the NH_4Cl removal technique (Roos & Boron, 1981) was 43 ± 5 mM/pH, the maximal rate of H^+ flux via the luminal antiporter was 116 ± 9 mM/min whereas the maximal rate of H^+

flux via the basolateral antiporter was 30 ± 2 mM/min.

Discussion

The results of the present study demonstrate that the outer medullary thin descending limb of long-loop nephrons, possesses an apical and basolateral Na^+/H^+ antiporter. Both antiporters function under steady-state conditions, however, the flux of protons across the apical antiporter exceeds the basolateral proton flux. The rate of H^+ (and Na^+) transport across each antiporter in vivo will be determined by the in vivo Na_i^+/Na_o^+ and H_i^+/H_o^+ concentration gradients across each cell membrane (where Na_i^+ and H_i^+ represent the intracellular concentration of Na^+ and H^+, respectively, and Na_o^+ and H_o^+ represent extracellular ion concentrations) and by the kinetic properties of each antiporter. In vivo, it is likely that the luminal and basolateral Na^+ concentration exceed the intracellular Na^+ concentration. Both the luminal and/or the basolateral antiporter could potentially mediate cellular H^+ efflux under steady-state conditions thereby generating intracellular base. Whether net base is generated intracellularly for subsequent transport across the luminal and/or basolateral membrane will depend on whether the rate of intracellular base generation by both antiporters exceeds the rate of metabolic H^+ production and passive H^+ influx in this tubule segment. Further studies are being performed to describe the HCO_3^-/OH^--dependent pH_i regulatory processes in this segment.

The finding that following acute intracellular acid loading, pH_i failed to recover in the absence of Na^+ (lumen, bath) indicates that the outer medullary tDL of long-loop nephrons lacks a Na^+-independent plasma membrane H^+-ATPase, which contributes importantly to regulation of pH_i. In contrast, the rabbit S_3 proximal tubule (the nephron segment immediately proximal to the tDL), has been recently shown to possess a plasma membrane H^+-ATPase, which regulates pH_i following acute intracellular acid loading in the absence of Na^+ (Kurtz, 1987). A recent study of the rat tDL utilizing a specific antibody has found H^+-ATPase activity on both the apical and basolateral cell membranes (S. Gluck, *personal communication*). Species and methodological differences may account for the discrepancy between these histochemical results and the results of the present study.

We have recently reported that the rabbit S_3 proximal straight tubule possesses a basolateral Na^+/H^+ antiporter (Kurtz, 1988). In that study, no distinction was made between short-loop and long-loop nephrons, suggesting that basolateral Na^+/H^+ antiport activity is present in both nephron populations. The results of the present study indicate that basolateral antiporter activity extends into the inner stripe of the outer medulla in long-loop tDL's. Whether the outer medullary tDL of short-loop nephrons and the inner medullary tDL possess a basolateral (or luminal) Na^+/H^+ antiporter is presently unknown. The basolateral antiporter in long-loop tDL's mediates cellular H^+ efflux in the steady state and participates in pH_i regulation following acute intracellular acid loading. Whether the basolateral antiporter in long-loop tDL's also functions in vivo as a Na^+-NH_4^+ exchanger as previously hypothesized in the S_3 proximal tubule (Kurtz, 1988) requires further study.

Both the apical and basolateral amiloride-inhibitable Na^+-dependent rates of pH_i recovery were greater when pH_i was decreased to ≈5.9. This finding suggests that either i) apical and basolateral Na^+/H^+ antiport activity is increased following intracellular acidification; ii) the K_i of amiloride is lower when pH_i is decreased; or iii) the rabbit tDL of long-loop nephrons possesses apical and basolateral Na^+-dependent amiloride (1 mM) insensitive $H^+/OH^-/HCO_3^-$ coupled transport pathways, which become quantitatively less important as pH_i is decreased. Further studies are needed to distinguish between these possibilities.

The failure to demonstrate net transepithelial volume reabsorption in the rabbit outer medullary tDL (Kokko, 1970) is of interest given the finding in the present study that the luminal Na^+/H^+ antiporter functions in the steady state and the recent study by Guggino and Lopez (1988) demonstrating functional basolateral Na^+/K^+-ATPase activity in this segment. It is clear that studies measuring rates of transepithelial HCO_3^- transport are required.

Although it is speculative, we would like to suggest a possible additional role for the luminal Na^+/H^+ antiporter in the renal countercurrent of transport of ammonia. Micropuncture studies of long-loop nephrons have revealed that the luminal pH at the bend of Henle's loop is approximately 7.39 (Buerkert, Martin & Trigg, 1983; Dubose et al., 1983). The increase in pH between the distal superficial proximal tubule micropuncture site, approximately 6.8 (Buerkert et al., 1983; Dubose et al., 1983) and the bend of the loop has been attributed to water absorption from the lumen of the tDL raising the luminal dissolved CO_2 concentration. The elevation of luminal dissolved CO_2 would result in a flux of CO_2 out of the lumen thereby elevating the

luminal pH. The delivery of ammonia to the bend of Henle's loop of deep nephrons exceeds the delivery of ammonia to the late superficial proximal tubule micropuncture site (Buerkert, Martin & Trigg, 1982). Assuming that the ammonia production rate in the superficial proximal tubule and deep nephrons is comparable, these results suggest that ammonia is secreted into the tubule lumen between the late superifical proximal tubule and the bend of Henle's loop (Buerkert et al., 1982). Ammonia secretion (resulting from the passive luminal entry of NH_3) has been demonstrated in the in vitro perfused rabbit S_2 and S_3 proximal straight tubule (Kurtz et al., 1986; Garvin, Burg & Knepper, 1987). It has been suggested that ammonia secretion may also occur in the tDL (Good & Knepper, 1985). Water absorption from the lumen of the outer medullary tDL (the nephron segment immediately distal to the S_3 proximal tubule) would not only increase the luminal dissolved CO_2 concentration but also the luminal NH_3 concentration. An elevation of luminal pH[1] as a result of dissolved CO_2 efflux would shift the luminal NH_3-NH_4^+ reaction towards NH_3, thereby further increasing the luminal NH_3 concentration. However, elevation of the luminal NH_3 concentration as a result of water absorption and CO_2 removal in the outer medullary tDL would decrease the transtubular gradient for luminal NH_3 influx in this segment and thereby inhibit outer medullary ammonia recycling. It is possible that an important function of the luminal Na^+/H^+ antiporter in the outer medullary tDL of long-loop nephrons is to prevent the luminal pH from increasing, thereby maintaining a lower luminal NH_3 concentration profile, which would favor the passive luminal influx of NH_3.

In summary, the rabbit outer medullary thin descending limb of long-loop nephrons possess apical and basolateral Na^+/H^+ antiporters. The flux of H^+ (and therefore Na^+) on the apical antiporter exceeds the flux on the basolateral antiporter under steady-state conditions and following acute intracellular acid loading. No evidence was found for a plasma membrane H^+-ATPase, which regulates pH_i following acute intracellular acid loading in this tubule segment.

[1] Luminal pH would increase as a result of water absorption only if the efflux of dissolved CO_2 exceeded the luminal NH_3 efflux. The rate of passive CO_2 and NH_3 flux in vivo would depend on the transtubular concentration gradients and the transepithelial permeability of these species.

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