Proc. Natl. Acad. Sci. USA
Vol. 92, pp. 12525–12529, December 1995
Physiology

Basolateral membrane Na^+/H^+ exchange enhances HCO_3^- absorption in rat medullary thick ascending limb: Evidence for functional coupling between basolateral and apical membrane Na^+/H^+ exchangers

(kidney/intracellular pH/amiloride/renal acidification/cross-talk)

DAVID W. GOOD*, THAMPI GEORGE, AND BRUNS A. WATTS III

Departments of Medicine and Physiology & Biophysics, University of Texas Medical Branch, Galveston, TX 77555

Communicated by Robert W. Berliner, Yale University, New Haven, CT, August 9, 1995

ABSTRACT **The role of basolateral membrane Na^+/H^+ exchange in transepithelial HCO_3^- absorption (J_{HCO_3}) was examined in the isolated, perfused medullary thick ascending limb (MTAL) of the rat. In Na^+-free solutions, addition of Na^+ to the bath resulted in a rapid, amiloride-sensitive increase in intracellular pH. In MTALs perfused and bathed with solutions containing 146 mM Na^+ and 25 mM HCO_3^-, bath addition of amiloride (1 mM) or 5-(*N*-ethyl-*N*-isopropyl) amiloride (EIPA, 50 μM) reversibly inhibited J_{HCO_3} by 50%. Evidence that the inhibition of J_{HCO_3} by bath amiloride was the result of inhibition of Na^+/H^+ exchange included the following: (*i*) the IC_{50} for amiloride was 5–10 μM, (*ii*) EIPA was a 50-fold more potent inhibitor than amiloride, (*iii*) the inhibition by bath amiloride was Na^+ dependent, and (*iv*) significant inhibition was observed with EIPA as low as 0.1 μM. Fifty micromolar amiloride or 1 μM EIPA inhibited J_{HCO_3} by 35% when added to the bath but had no effect when added to the tubule lumen, indicating that addition of amiloride to the bath did not directly inhibit apical membrane Na^+/H^+ exchange. In experiments in which apical Na^+/H^+ exchange was assessed from the initial rate of cell acidification following luminal EIPA addition, bath EIPA secondarily inhibited apical Na^+/H^+ exchange activity by 46%. These results demonstrate that basolateral membrane Na^+/H^+ exchange enhances transepithelial HCO_3^- absorption in the MTAL. This effect appears to be the result of cross-talk in which an increase in basolateral membrane Na^+/H^+ exchange activity secondarily increases apical membrane Na^+/H^+ exchange activity.**

Na^+/H^+ exchangers have been identified in both apical and basolateral membranes of epithelial cells. Basolateral membrane Na^+/H^+ exchange (NHE1 isoform) is amiloride-sensitive and is involved in housekeeping functions such as regulation of intracellular pH (pH_i) and cell volume (1–7). Apical membrane Na^+/H^+ exchange (NHE3 isoform in kidney and intestine) is relatively amiloride-resistant and mediates transepithelial reabsorption of NaCl and $NaHCO_3$ (2–5, 7–10). Functional studies have shown that apical and basolateral membrane Na^+/H^+ exchangers coexist in renal tubule cells (1, 3, 5, 11–13). However, little is known about their functional interactions.

In the rat kidney, the thick ascending limb of the loop of Henle reabsorbs a significant fraction of filtered HCO_3^- (8, 14). This HCO_3^- absorption is achieved by secretion of H^+ across the apical membrane and efflux of HCO_3^- across the basolateral membrane (8). In the medullary thick ascending limb (MTAL), H^+ secretion is mediated virtually completely by apical membrane Na^+/H^+ exchange (8). Thus, the rate of transepithelial HCO_3^- absorption serves as a measure of apical Na^+/H^+ exchange activity under steady-state conditions. Transport mechanisms that may contribute to basolateral HCO_3^- efflux include Na^+–HCO_3^- cotransport, K^+–HCO_3^- cotransport, and Cl^-/HCO_3^- exchange (8).

In addition to HCO_3^- transport pathways, the basolateral membrane of many nephron segments also contains a Na^+/H^+ exchanger (1, 6, 12). The role of this exchanger in transcellular HCO_3^- absorption is largely unknown. In principle, basolateral membrane Na^+/H^+ exchange should decrease the efficiency of HCO_3^- absorption because it opposes net base efflux. Although this notion is widely believed (1, 2, 7, 11, 12), there have been no studies of the role of basolateral Na^+/H^+ exchange in transepithelial HCO_3^- absorption. The present experiments were designed to examine the influence of basolateral membrane Na^+/H^+ exchange on HCO_3^- absorption in the MTAL of the rat. The results show that, contrary to the prevailing view that this transporter opposes HCO_3^- absorption, basolateral membrane Na^+/H^+ exchange markedly enhances transepithelial HCO_3^- absorption in the MTAL. This enhancement appears to be the result of an effect of basolateral membrane Na^+/H^+ exchange to secondarily increase the activity of apical membrane Na^+/H^+ exchange.

METHODS

Tubule Perfusion. MTALs from male Sprague–Dawley rats (50–80 g; Taconic) were isolated and perfused *in vitro* (14–17). In most experiments, the tubules were perfused and bathed in control solution that contained (in mM) 146 Na^+, 4 K^+, 122 Cl^-, 25 HCO_3^-, 2.0 Ca^{2+}, 1.5 Mg^{2+}, 2.0 phosphate, 1.2 SO_4^{2-}, 1.0 citrate, 2.0 lactate, and 5.5 glucose (equilibrated with 95% O_2/5% CO_2; pH 7.45 at 37°C). Modifications of this solution are described in *Results*. In some experiments (see Fig. 1), tubules were perfused and bathed in Na^+-free Hepes solution that contained (in mM) 145 *N*-methyl-D-glucamine ($NMDG^+$), 4 K^+, 147 Cl^-, 2.0 Ca^{2+}, 1.5 Mg^{2+}, 2.0 phosphate, 1.0 SO_4^{2-}, 2.0 lactate, 5.5 glucose, and 5 Hepes (equilibrated with 100% O_2 and titrated to pH 7.4). All bath solutions contained 0.2% fatty acid-free bovine serum albumin (Sigma).

Measurement of Net HCO_3^- Absorption. Transepithelial HCO_3^- absorption was studied as described (15, 18). The tubules were equilibrated for 20–30 min at 37°C in control solution and the luminal perfusion rate was adjusted to 1.5–1.9 nl/(min·mm). Two to four 10-min tubule fluid samples were

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: MTAL, medullary thick ascending limb; $NMDG^+$, *N*-methyl-D-glucamine; EIPA, 5-(*N*-ethyl-*N*-isopropyl)amiloride; pH_i, intracellular pH.

*To whom reprint requests should be addressed at: 4.200 John Sealy Hospital 0562, University of Texas Medical Branch, 301 University Boulevard, Galveston, TX 77555.

collected for each experimental period (control, experimental, recovery). The length of the perfused segments was 0.57 ± 0.01 mm. The absolute rate of HCO_3^- absorption was calculated from the fluid flow rate and the difference between total CO_2 concentrations in perfused and collected fluids (15).

Measurement of pH_i. pH_i was measured in isolated, perfused MTALs by use of the pH-sensitive dye 2′, 7′-bis(carboxyethyl)-5(6)-carboxyfluorescein (BCECF) and a microfluorometer coupled to the perfusion apparatus (16, 17). Intracellular dye was excited at 500 nm and 440 nm with either a manually controlled filter exchange system (16) (see Fig. 1) or a computer-controlled spectrofluorometer (Spex Industries, Metuchen, NJ) (see Fig. 5). Emitted light was monitored at 530 nm. pH_i calibration of the fluorescence excitation ratio (F_{500}/F_{440}) was obtained by use of high-K^+-nigericin standards (16, 17).

Apical membrane Na^+/H^+ exchange activity [$J_{Na/H}$, pmol/(min·mm)] was assessed by measuring the equivalent net H^+ influx following rapid addition of 5-(*N*-ethyl-*N*-isopropyl)amiloride (EIPA) to the tubule lumen (see *Results*). In this analysis, $J_{Na/H} = dpH_i/dt \times \beta_T \times V$, where dpH_i/dt is the initial rate of cell acidification following luminal EIPA addition (pH units/min), β_T is the total intracellular buffering power (mM/pH unit), and V is cell volume/mm of tubule length (nl/mm). β_T is the sum of the intrinsic intracellular buffering power, measured previously as a function of pH_i (17), and buffering power due to HCO_3^-/CO_2, computed as $2.3[HCO_3^-]_i$ (19). V averages 0.30 nl/mm (17, 18). Solutions containing EIPA, amiloride, $NMDG^+$, and BCECF were prepared as described (16, 17).

Statistical Analysis. Results are presented as mean ± SE. Differences between means were evaluated by paired Student's *t* test, with $P < 0.05$ considered statistically significant.

RESULTS

Evidence for Basolateral Membrane Na^+/H^+ Exchange. MTALs were perfused and bathed in Na^+-free Hepes solution (pH 7.4) with furosemide (0.1 mM) and amiloride (1 mM) present in the luminal perfusate to inhibit apical Na^+ entry pathways (Fig. 1). Addition of 145 mM Na^+ to the bath (arrow) resulted in a rapid cell alkalinization (solid line). The initial rate of pH_i increase was inhibited 85% by 50 μM bath EIPA (broken line), a potent Na^+/H^+ exchange inhibitor [2.1 ± 0.1 pH units/min (Na^+) vs 0.3 ± 0.1 pH units/min (Na^+ plus EIPA), $n = 3$; $P < 0.001$]. Similar results were obtained with solutions that contained no Cl^- or organic anions. In a second protocol, MTALs were perfused and bathed in control solution containing 146 mM Na^+ and 25 mM HCO_3^- (pH 7.4). Under these conditions, addition of 1 mM amiloride to the bath decreased steady-state pH_i reversibly from 7.16 ± 0.03 to 7.09 ± 0.04 ($n = 7$; $P < 0.05$), and addition of 1 μM EIPA to the bath decreased pH_i from 7.10 ± 0.03 to 7.05 ± 0.02 ($n = 5$; $P < 0.025$). These results demonstrate that the MTAL possesses a basolateral membrane Na^+/H^+ exchanger that is active at the resting pH_i (7.1–7.2) (16).

Bath Amiloride Inhibits HCO_3^- Absorption. In MTALs perfused and bathed in control solution, addition of 1 mM amiloride or 50 μM EIPA to the bath inhibited HCO_3^- absorption reversibly by 53%, from 14.9 ± 0.7 to 7.0 ± 1.1 pmol/(min·mm) ($n = 5$; $P < 0.005$) (Fig. 2). Previous work has shown that [Arg^8]vasopressin inhibits HCO_3^- absorption by increasing cAMP (15). In four additional tubules studied with 0.1 nM [Arg^8]vasopressin in the bath solution, addition of 1 mM amiloride or 50 μM EIPA to the bath decreased HCO_3^- absorption by 56%, from 8.7 ± 0.5 to 3.8 ± 0.7 pmol/(min·mm) ($P < 0.005$). Thus, the effects of bath amiloride and [Arg^8]vasopressin were additive.

Further experiments were performed with 1 μM amiloride and 1 μM EIPA. This concentration was chosen to identify a functional effect on Na^+/H^+ exchange because, at physiological Na^+ concentrations, Na^+/H^+ exchangers are negligibly affected by 1 μM amiloride but are inhibited significantly by 1 μM EIPA (2). Addition of 1 μM EIPA to the bath inhibited HCO_3^- absorption by 35%, from 14.3 ± 1.0 to 9.4 ± 0.8 pmol/(min·mm) ($n = 4$; $P < 0.005$), an effect similar to that observed with 50 μM EIPA or 1 mM amiloride. In contrast, 1 μM amiloride decreased HCO_3^- absorption by only 6%, from 15.5 ± 0.7 to 14.5 ± 0.7 pmol/(min·mm) ($n = 4$; $P < 0.05$). These results are consistent with the known sensitivity of Na^+/H^+ exchange to the two inhibitors.

The dependence of HCO_3^- absorption on the concentration of bath amiloride or EIPA is summarized in Fig. 3. Three results are noteworthy. (*i*) The half-maximal inhibitory concentration (IC_{50}) for amiloride was 5–10 μM. (*ii*) HCO_3^- absorption was ≈50 times more sensitive to EIPA than to amiloride. (*iii*) Significant inhibition of HCO_3^- absorption was observed with as little as 0.1 μM EIPA. Taken together, these results support the conclusion that bath amiloride inhibits

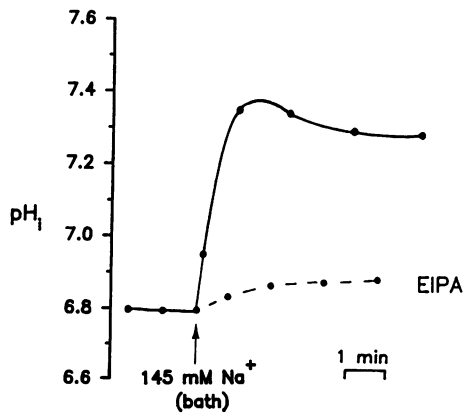

FIG. 1. Effect of bath Na^+ addition on pH_i. MTALs were perfused and bathed in Na^+-free Hepes solution (pH 7.4) in the absence (solid line) or presence (broken line) of 50 μM bath EIPA. Addition of 145 mM Na^+ to the bath (Na^+ replaced $NMDG^+$) caused a rapid increase in pH_i (arrow). EIPA inhibited the initial rate of pH_i recovery by 85%. Each tracing shows the results of a single experiment. Data points were obtained from F_{500}/F_{440} fluorescence ratios (16). Lines were drawn by eye.

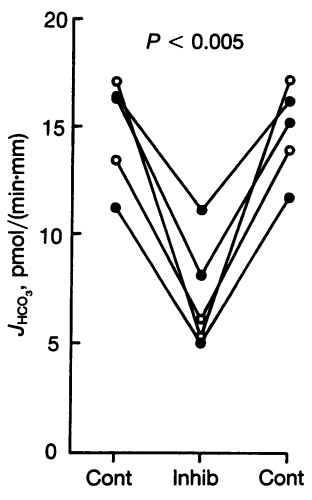

FIG. 2. Effects of amiloride (1 mM) (●) and EIPA (50 μM) (○) in the bath on HCO_3^- absorption by rat MTALs. Circles represent mean values for single tubules; lines connect paired measurements made in the same tubule. *P* value is for paired *t* test. Mean values are in the text. Cont, control; Inhib, with inhibitor (amiloride or EIPA).

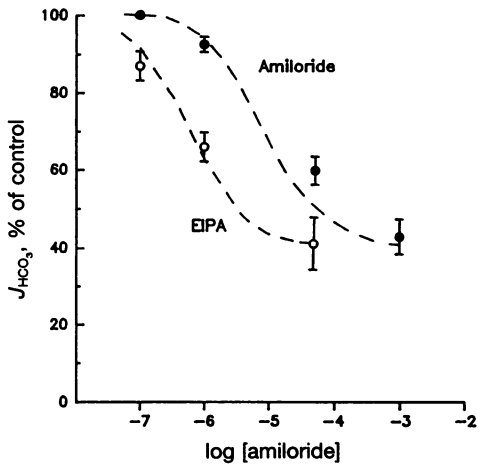

FIG. 3. Inhibition of HCO_3^- absorption by bath amiloride (●) and EIPA (○) in rat MTALs. One inhibitor concentration was studied per tubule. HCO_3^- absorption rate in the presence of inhibitor is plotted as percent of the control rate measured in the same tubule. Data points are means ± SE for three to six tubules. Lines were drawn by eye. All concentrations inhibited J_{HCO_3} significantly ($P < 0.05$) except 0.1 μM amiloride (P not significant).

HCO_3^- absorption through inhibition of Na^+/H^+ exchange (see *Discussion*).

Addition of Amiloride to the Bath Does Not Directly Inhibit Apical Membrane Na^+/H^+ Exchange. Amiloride or EIPA added to the bath solution could inhibit HCO_3^- absorption by entering the tubule lumen or the cells and directly inhibiting the apical membrane Na^+/H^+ exchanger. Two series of experiments were performed to assess this possibility. The first series was based on previous observations that basolateral membrane Na^+/H^+ exchange is more sensitive to amiloride than apical membrane Na^+/H^+ exchange (2–5). Addition of 50 μM amiloride to the tubule lumen had no effect on HCO_3^- absorption [11.6 ± 0.4 pmol/(min·mm) (control) vs 11.3 ± 0.4 pmol/(min·mm) (lumen amiloride); $n = 4$]. In contrast, addition of 50 μM amiloride to the bath, in the continued presence of amiloride in the lumen, inhibited HCO_3^- absorption by 38%, from 11.1 ± 0.5 to 6.8 ± 0.4 pmol/(min·mm) ($n = 3$; $P < 0.005$) (Fig. 4*A*). Similar results were obtained with 1 μM EIPA, which inhibited HCO_3^- absorption by 35% when added to the bath (see above) but had no effect when added directly to the tubule lumen [13.2 ± 1.2 pmol/(min·mm) (control) vs 13.2 ± 1.3 pmol/(min·mm) (luminal EIPA), $n = 3$; P not significant].

The second series of experiments was based on the premise that amiloride or EIPA in the bath would have little effect on basolateral Na^+/H^+ exchange in the absence of bath Na^+ but could still enter the tubule lumen or cells to inhibit apical Na^+/H^+ exchange. MTALs were perfused with control solution (containing 25 mM HCO_3^- and 146 mM Na^+). The bath solution was identical except that Na^+ was replaced completely with $NMDG^+$. Under these conditions, addition of 50 μM EIPA to the bath had no effect on HCO_3^- absorption (Fig. 4*B*). In contrast, in the continued presence of bath EIPA, addition of 50 μM EIPA to the tubule lumen inhibited HCO_3^- absorption reversibly by 85% [12.2 ± 1.0 pmol/(min·mm) (bath EIPA) vs 1.8 ± 0.4 pmol/(min·mm) (bath and luminal EIPA), $n = 3$; $P < 0.005$]. These results indicate that amiloride and EIPA in the bath do not inhibit HCO_3^- absorption by direct inhibition of apical membrane Na^+/H^+ exchange and that the effect of basolateral EIPA to inhibit HCO_3^- absorption requires Na^+ in the same solution.†

Bath Amiloride Secondarily Inhibits Apical Membrane Na^+/H^+ Exchange. In the MTAL, HCO_3^- absorption is mediated virtually completely by apical membrane Na^+/H^+ exchange (8, 17, 18). Thus, we tested whether inhibition of basolateral membrane Na^+/H^+ exchange secondarily inhibits apical membrane Na^+/H^+ exchange. Apical Na^+/H^+ exchange activity was assessed by measuring the initial rate of cell acidification following rapid addition of 50 μM EIPA to the tubule lumen. The basis for this approach is that prior to luminal EIPA addition, when pH_i is stable, H^+ extrusion via the apical Na^+/H^+ exchanger balances other transport and metabolic processes tending to acid-load the cells (17, 20). When the apical exchanger is inhibited, the initial rate of cell

†The basal rate of HCO_3^- absorption in tubules studied in the Na^+-free bath is similar to rates observed in the presence of Na^+. This most likely occurs because bath Na^+ removal has additional effects not observed with amiloride addition that tend to increase HCO_3^- absorption, thereby offsetting the effect of inhibiting basolateral Na^+/H^+ exchange. These effects may include an increase in the driving force for Na^+-coupled basolateral HCO_3^- efflux and a decrease in cell $[Na^+]$ that accelerates apical Na^+/H^+ exchange.

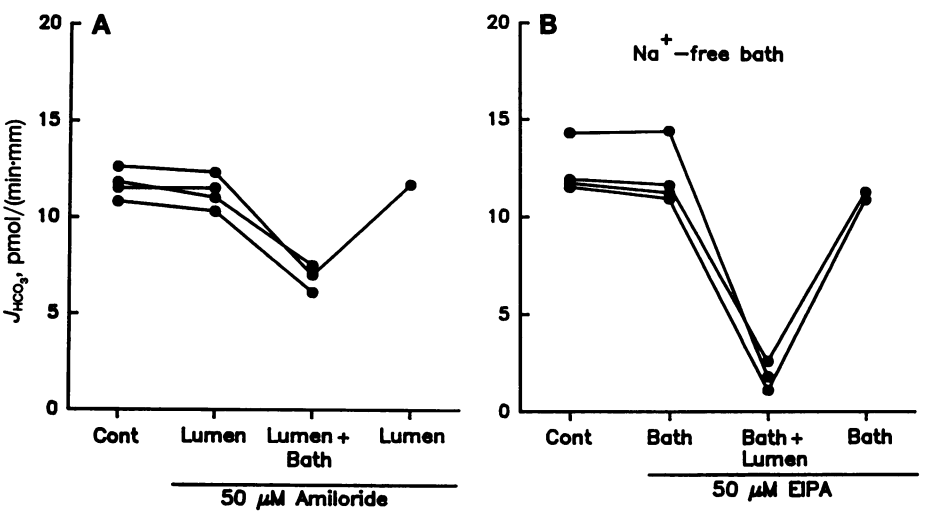

FIG. 4. Evidence that amiloride addition to the bath does not directly inhibit apical membrane Na^+/H^+ exchange. (*A*) MTALs were perfused and bathed in control solution (containing 146 mM Na^+ and 25 mM HCO_3^-). Addition of 50 μM amiloride to the lumen had no effect on HCO_3^- absorption. Addition of 50 μM amiloride to the bath in the continued presence of luminal amiloride inhibited HCO_3^- absorption by 35%. (*B*) MTALs were perfused with control solution; bath Na^+ was replaced completely with $NMDG^+$ throughout the experiment. Addition of 50 μM EIPA to the bath had no effect on HCO_3^- absorption in the absence of bath Na^+. Addition of 50 μM EIPA to the lumen in the continued presence of bath EIPA inhibited HCO_3^- absorption by 85%. Circles and lines are as in Fig. 2. Mean values are in text.

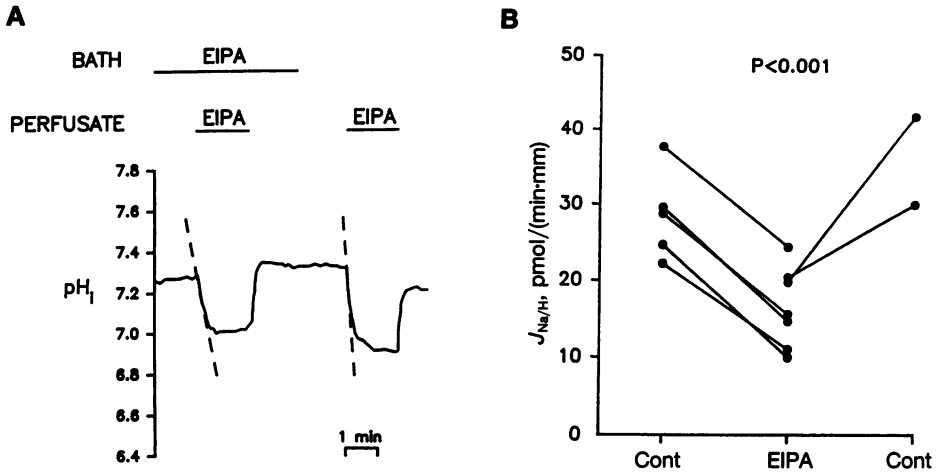

FIG. 5. Bath EIPA secondarily inhibits apical membrane Na^+/H^+ exchange. (*A*) A MTAL was perfused and bathed in control solution (containing 146 mM Na^+ and 25 mM HCO_3^-). Apical Na^+/H^+ exchange activity was assessed in the presence and absence of 50 μM bath EIPA from the initial rate of cell acidification (broken lines) following luminal addition of 50 μM EIPA (see text). (*B*) Effect of bath EIPA on apical membrane Na^+/H^+ exchange activity in seven experiments similar to *A*. Circles, lines, and *P* value are as in Fig. 2.

acidification provides an estimate of the steady-state rate at which apical Na^+/H^+ exchange operates to balance background acid loading. Fig. 5*A* shows the results of such an experiment. pH_i was monitored in MTAL perfused and bathed in control solution (containing 25 mM HCO_3^- and 146 mM Na^+; pH 7.4). EIPA (50 μM) initially was present in the bath solution and steady-state pH_i was $\approx$7.25. Addition of 50 μM EIPA to the luminal perfusate decreased pH_i due to inhibition of apical membrane Na^+/H^+ exchange (8, 17). pH_i recovered when EIPA was removed from the luminal fluid. EIPA was then removed from the bath solution and the pH_i response to luminal EIPA addition was repeated. The results show that the initial rate of intracellular acidification in response to luminal EIPA addition (broken lines) was decreased significantly in the presence of bath EIPA. Similar results were obtained when the order of the experimental conditions was reversed. In seven such experiments (Fig. 5*B*), apical membrane Na^+/H^+ exchange activity was decreased by 46% in the presence of 50 μM bath EIPA. These results suggest that inhibition of basolateral membrane Na^+/H^+ exchange results secondarily in inhibition of apical membrane Na^+/H^+ exchange.

DISCUSSION

The results demonstrate that inhibition of basolateral membrane Na^+/H^+ exchange markedly inhibits HCO_3^- absorption in the MTAL of the rat. Thus, basolateral Na^+/H^+ exchange activity appears to be important to sustain high rates of HCO_3^- absorption in this segment. The effect of basolateral Na^+/H^+ exchange to accelerate HCO_3^- absorption is the result of functional coupling between basolateral and apical membrane Na^+/H^+ exchangers. These results suggest that the regulation of transepithelial HCO_3^- absorption can be achieved through regulation of basolateral, as well as apical, Na^+/H^+ exchange.

Bath Amiloride Inhibits HCO_3^- Absorption Through Inhibition of Basolateral Membrane Na^+/H^+ Exchange. Several observations support the view that the effect of bath amiloride to inhibit HCO_3^- absorption is due to inhibition of Na^+/H^+ exchange rather than to effects on other cellular processes. (*i*) EIPA was a 50-fold more potent inhibitor than amiloride, consistent with the known sensitivity of Na^+/H^+ exchange to these two compounds (2, 21, 22). (*ii*) The estimated IC_{50} for amiloride inhibition of HCO_3^- absorption was similar to values reported for inhibition of Na^+/H^+ exchange in the MTAL and many other systems (2, 4, 21, 23). (*iii*) The inhibition was Na^+ dependent. (*iv*) Inhibition of HCO_3^- absorption was observed with 0.1–1.0 μM EIPA. At these low concentrations, EIPA significantly inhibits Na^+/H^+ exchange but has no detectable effect on other cellular processes, including Na^+,K^+-ATPase activity, Na^+/Ca^{2+} exchange, Na^+ or Ca^{2+} channels, protein synthesis, DNA replication/transcription, oxidative metabolism, and ATP/ADP content (21, 22, 24, 25). Possible effects of EIPA or amiloride to inhibit protein kinases also are unlikely, since inhibition of cAMP-dependent protein kinase or protein-tyrosine kinase stimulates HCO_3^- absorption (15, 18), whereas inhibition of protein kinase C has no effect (26). High concentrations of amiloride (0.5–1.0 mM) also have no effect on thick ascending limb HCO_3^- efflux pathways, including basolateral membrane Na^+–HCO_3^- cotransport (23, 27, 28).

The possibility that bath amiloride or EIPA inhibits HCO_3^- absorption by entering the tubule lumen or cell and inhibiting the apical membrane Na^+/H^+ exchanger can be ruled out for several reasons. (*i*) Fifty micromolar amiloride and 1 μM EIPA markedly inhibited HCO_3^- absorption when added to the bath but had no effect when added directly to the tubule lumen. (*ii*) The IC_{50} for inhibition of HCO_3^- absorption by bath amiloride is in close agreement with values expected for the amiloride-sensitive Na^+/H^+ exchanger localized to basolateral membranes (NHE1) but is one order of magnitude below the IC_{50} needed for inhibition of the amiloride-resistant exchanger localized in apical membranes (NHE3) (2, 4, 29). (*iii*) In the absence of bath Na^+, addition of 50 μM EIPA to the bath had no effect on HCO_3^- absorption, whereas concurrent addition to the tubule lumen inhibited HCO_3^- absorption by 85%. (*iv*) Na^+/H^+ exchange is not inhibited by amiloride from the cytoplasmic surface (30, 31). Taken together, these findings suggest that the inhibitory effect is on basolateral rather than apical membrane Na^+/H^+ exchange. We conclude, therefore, that the inhibition of HCO_3^- absorption by bath amiloride is mediated through inhibition of basolateral membrane Na^+/H^+ exchange.

Functional Coupling Between Basolateral and Apical Membrane Na^+/H^+ Exchangers. The present study demonstrates that the MTAL of the rat contains a basolateral membrane Na^+/H^+ exchanger, consistent with previous results in rat cortical thick ascending limb (27) and mouse MTAL (13). Current evidence suggests that the dominant exchanger isoforms in the thick ascending limb are NHE1 in the basolateral membrane and NHE3 in the apical membrane (6, 18, 32). It is widely assumed for renal epithelia that basolateral membrane Na^+/H^+ exchange decreases the efficiency of transepithelial

HCO_3^- absorption by decreasing net basolateral base efflux and/or by increasing cell $[Na^+]$ or pH and secondarily inhibiting apical membrane Na^+/H^+ exchange (1, 2, 11, 12). Our results show, however, that basolateral membrane Na^+/H^+ exchange enhances HCO_3^- absorption in the MTAL and that this effect can be explained by an increase in apical membrane Na^+/H^+ exchange activity. The influence of basolateral membrane Na^+/H^+ exchange on transepithelial HCO_3^- absorption is thus the result of cross-talk in which a primary increase in Na^+/H^+ exchange across the basolateral membrane results secondarily in an increase in Na^+/H^+ exchange across the apical membrane. Our results also provide functional evidence that the apical and basolateral membrane Na^+/H^+ exchangers identified in the MTAL are located in the same cell.

The mechanism of the functional coupling between the exchangers was not identified in our experiments. Changes in the apical membrane Na^+/H^+ exchange rate could result from changes in (*i*) the net driving force, (*ii*) the turnover number (i.e., the "activity" of individual transporters), and/or (*iii*) the number of copies of the transporter (due to insertion/retrieval or activation/inactivation). Our results are not consistent with the first possibility, because inhibition of basolateral Na^+/H^+ exchange increases intracellular $[H^+]$ and presumably decreases intracellular $[Na^+]$, changes that should stimulate apical Na^+/H^+ exchange. In contrast, the apical Na^+/H^+ exchange rate decreases. Our results are consistent with a change in transporter activity or transporter number via an intracellular signaling mechanism. This signaling is unlikely to involve cAMP, because the inhibition by bath amiloride was additive to inhibition by 0.1 nM [Arg^8]vasopressin, which produces maximal cAMP-dependent inhibition of HCO_3^- absorption (15). A possibility is that the coupling may be mediated by a change in cell volume. Basolateral membrane Na^+/H^+ exchange mediates hypertonic regulatory volume increase in the mouse MTAL (33), and hyperosmolality inhibits apical membrane Na^+/H^+ exchange and HCO_3^- absorption in the rat MTAL (8, 17, 18). If basolateral Na^+/H^+ exchange contributes normally to the maintenance of cell volume under isosmotic conditions, then inhibition of this exchanger could result in cell shrinkage that secondarily inhibits the apical Na^+/H^+ exchanger.‡ Further work is needed to test this hypothesis.

Although the present results provide evidence for functional coupling between Na^+/H^+ exchangers, apical and basolateral membrane Na^+/H^+ exchange also can be regulated differentially. In the MTAL, hyperosmolality stimulates basolateral Na^+/H^+ exchange (33) but inhibits apical Na^+/H^+ exchange (17). Differential regulation of apical and basolateral exchangers also has been observed in the MTAL with [Arg^8]vasopressin and in cultured renal epithelial cells with [Arg^8]vasopressin, parathyroid hormone, and calcitonin (5, 13, 34). These data may reflect different regulatory properties of NHE isoforms and/or cell-specific regulation of individual isoforms (2). We do not know the physiologic stimuli that may initiate the coordinated operation of the exchangers observed in the present study. Coordinated regulation of the exchangers may be important for controlling uptake of Na^+ into the MTAL cells in the high-Na^+ environment of the renal medulla, where lumen and interstitial Na^+ concentrations vary widely with changes in salt and water balance and may exceed 300 mM (35). The functional coupling also may provide a mechanism by which changes in interstitial fluid composition or regulatory factors such as growth factors or cytokines may modulate transepithelial HCO_3^- absorption through regulation of basolateral membrane Na^+/H^+ exchange. Further work is needed to identify stimuli that initiate cross-talk between the exchangers and to assess the extent to which the physiologic regulation of luminal acidification may be achieved through control of basolateral membrane Na^+/H^+ exchange.

‡No change in cell volume was observed visually in the perfusion microscope with bath amiloride. However, this assessment is not sufficiently accurate to detect small (10–20%) changes in volume that may be significant physiologically. A small decrease in cell volume with bath EIPA, if it occurs, would not significantly influence the calculated $J_{Na/H}$ values (Fig. 5) because (*i*) β_i varies proportionally and inversely with cell volume in the MTAL (17) and (*ii*) bath EIPA had a negligible effect on buffering power due to HCO_3^-/CO_2.

We thank L. Reuss for critical reading of the manuscript. This work was supported by Grant DK38217 from the National Institutes of Health.

1. Preisig, P. A. & Alpern, R. J. (1991) *Kidney Int.* **39,** 1077–1086.
2. Noel, J. & Pouyssegur, J. (1995) *Am. J. Physiol.* **268,** C283–C296.
3. Haggerty, J. G., Agarwal, N., Reilly, R. F., Adelberg, E. A. & Slayman, C. W. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 6797–6801.
4. Knickelbein, R. G., Aronson, P. S. & Dobbins, J. W. (1990) *Am. J. Physiol.* **259,** G802–G806.
5. Mrkic, B., Fargo, J., Murer, H. & Helmle-Kolb, C. (1992) *J. Membr. Biol.* **130,** 205–217.
6. Biemesderfer, D., Reilly, R. F., Exner, M., Igarashi, P. & Aronson, P. S. (1992) *Am. J. Physiol.* **263,** F833–F840.
7. Bookstein, C., DePaoli, A. M., Xie, Y., Niu, P., Musch, M. W., Rao, M. C. & Chang, E. B. (1994) *J. Clin. Invest.* **93,** 106–113.
8. Good, D. W. (1993) *Semin. Nephrol.* **13,** 225–235.
9. Alpern, R. J., Yamaji, Y., Amemiya, M., Cano, A., Preisig, P. A., Miller, R. T. & Moe, O. W. (1995) *News Physiol. Sci.* **10,** 77–81.
10. Biemesderfer, D., Pizzonia, J., Abu-Alfa, A., Exner, M., Reilly, R., Igarashi, P. & Aronson, P. (1993) *Am. J. Physiol.* **265,** F736–F742.
11. Boron, W. F. & Boulpaep, E. L. (1983) *J. Gen. Physiol.* **81,** 29–52.
12. Mackovic-Basic, M. & Kurtz, I. (1990) *Semin. Nephrol.* **10,** 122–131.
13. Sun, A. M., Kikeri, D. & Hebert, S. C. (1992) *Am. J. Physiol.* **262,** F241–F247.
14. Good, D. W., Knepper, M. A. & Burg, M. B. (1984) *Am. J. Physiol.* **247,** F35–F44.
15. Good, D. W. (1990) *J. Clin. Invest.* **85,** 1006–1013.
16. Watts, B. A., III & Good, D. W. (1994) *J. Gen. Physiol.* **103,** 917–936.
17. Watts, B. A., III & Good, D. W. (1994) *J. Biol. Chem.* **269,** 20250–20255.
18. Good, D. W. (1995) *J. Biol. Chem.* **270,** 9883–9889.
19. Roos, A. & Boron, W. F. (1981) *Physiol. Rev.* **61,** 296–434.
20. Boyarsky, G., Ganz, M. B., Cragoe, E. J., Jr., & Boron, W. F. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 5921–5924.
21. Kleyman, T. R. & Cragoe, E. J., Jr. (1988) *J. Membr. Biol.* **105,** 1–21.
22. Grinstein, S., Rotin, D. & Mason, M. J. (1989) *Biochim. Biophys. Acta* **988,** 73–97.
23. Kikeri, D., Azar, S., Sun, A., Zeidel, M. L. & Hebert, S. C. (1990) *Am. J. Physiol.* **258,** F445–F456.
24. Soltoff, S. P., Cragoe, E. J., Jr., & Mandel, L. J. (1986) *Am. J. Physiol.* **250,** C744–C747.
25. Kaczorowski, G. J., Slaughter, R. S., King, V. F. & Garcia, M. L. (1989) *Biochim. Biophys. Acta* **988,** 287–302.
26. Good, D. W. & George, T. (1994) *J. Am. Soc. Nephrol.* **5,** 679.
27. Krapf, R. (1988) *J. Clin. Invest.* **82,** 234–241.
28. Leviel, F., Borensztein, P., Houillier, P., Paillard, M. & Bichara, M. (1992) *J. Clin. Invest.* **90,** 869–878.
29. Soleimani, M., Bookstein, C., McAteer, J. A., Hattabaugh, J., Bizal, G. L., Musch, M. W., Villereal, M., Rao, M. C., Howard, R. L. & Chang, E. B. (1994) *J. Biol. Chem.* **269,** 15613–15618.
30. Grinstein, S. & Smith, J. D. (1987) *J. Biol. Chem.* **262,** 9088–9092.
31. Desir, G. V., Cragoe, E. J., Jr., & Aronson, P. S. (1991) *J. Biol. Chem.* **266,** 2267–2271.
32. Borensztein, P., Froissart, M., Laghmani, K., Bichara, M. & Paillard, M. (1995) *Am. J. Physiol.* **268,** F1224–F1228.
33. Hebert, S. (1986) *Am. J. Physiol.* **250,** C920–C931.
34. Casavola, V., Reshkin, S. J., Murer, H. & Helmle-Kolb, C. (1992) *Pflugers Arch.* **420,** 282–289.
35. Johnston, P. A., Battilana, C. A., Lacy, F. B. & Jamison, R. L. (1977) *J. Clin. Invest.* **59,** 234–240.