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Research Article

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Apical Membrane Chloride/Base Exchange in the Rat Proximal Convoluted Tubule

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Abstract

To examine whether Cl/base exchange is present on the apical membrane of the proximal convoluted tubule, cell pH was measured fluorometrically in the in vivo microperfused rat proximal tubule with (2',7')-bis(carboxyethyl)-(5,6)-carboxyfluorescein. The effect of luminal chloride addition was examined in tubules perfused symmetrically with chloride-free solutions. In the absence of inhibitors, luminal chloride addition did not affect cell pH. However, after inhibition of basolateral membrane anion transport with peritubular 4-acetamido-4'-isothiocyano-(2,2')-disulfonic-stilbene (to amplify effects of apical membrane transport on cell pH), luminal chloride addition caused a small cell acidification (delta pH_i = 0.02). When 1 mM formate was added to the solutions, luminal chloride addition caused a larger change in cell pH (delta pH_i = 0.06) that was inhibited by (4,4')-diisothiocyano-(2,2')-disulfonic-stilbene. This stimulation of Cl/base exchange was not seen with 1 mM acetate addition. These results demonstrate apical membrane Cl/base exchange, a significant fraction of which is dependent on the presence of formate and probably represents Cl/formate exchange.

Introduction

Sodium chloride absorption across the proximal convoluted tubule (PCT)[1] occurs by paracellular and transcellular pathways (1–4). Transcellular NaCl absorption is electroneutral (3, 5, 6), but the exact apical and basolateral membrane mechanisms remain obscure. Three mechanisms by which sodium and chloride can cross the apical membrane have been proposed: direct coupling between sodium and chloride transport, parallel sodium and chloride conductances, and parallel Na/H and Cl/base exchangers (7).

Na/H antiport in parallel with Cl/base exchange would lead to absorption of NaCl and secretion of a proton and a base that would then neutralize each other. Inhibition of NaCl absorption by inhibitors of red blood cell anion exchange first suggested that parallel exchangers might mediate apical membrane NaCl absorption (8). Subsequent studies in isolated apical membrane vesicles have disagreed with respect to the presence or absence of Cl/OH exchange (9–15). Karniski and Aronson (16) found rapid Cl/formate exchange in these apical membranes.

The purpose of the present studies was to examine whether proximal tubular cells possess apical membrane Cl/base exchange. To accomplish this, cell pH was measured in the in vivo microperfused rat PCT using the pH-sensitive dye, (2',7')-bis(carboxyethyl)-(5,6)-carboxyfluorescein (BCECF). The results demonstrate Cl/base exchange, a significant fraction of which is dependent on the presence of formate.

Methods

Experiments were performed using male Wistar rats (Charles River Breeding Laboratories, Inc., Wilmington, MA) weighing 180–280 g. The rats were prepared for microperfusion as previously described (17). Briefly, rats were anesthetized with an intraperitoneal injection of Inactin (100–120 mg/kg) and placed on a heated table that maintained body temperature at 37°C. The left kidney was exposed by a flank incision and was immobilized in a lucite cup. The ureter was cannulated (PE-50) to ensure the free drainage of urine. Rats were infused intravenously with a bicarbonate Ringer's solution (105 nM NaCl, 25 mM $NaHCO_3$, 4 mM Na_2HPO_4, 5 mM KCl, 1 mM $MgSO_4$, 1.8 mM $CaCl_2$) at 3.2 ml/h during surgery and then 1.6 ml/h throughout the rest of the experiment. Proximal tubular transit time was measured following injection of 0.02 ml of 10% lissamine green dye intravenously, and only those kidneys in which transit time was < 11 s were accepted for study.

Pipettes were placed using a dissecting microscope (E. Leitz Inc., Rockleigh, NJ). Peritubular capillaries were perfused as previously described (18, 19) with a 12–14 μm tip pipette designed to allow rapid changes between two perfusion fluids. The lumen of a PCT was then perfused as previously described (19) using a technique of rapid retrograde perfusion similar to that described by Fromter and Gessner (20). First, tubules were perfused at 40 nl/min for 5–7 min using a thermally insulated microperfusion pump (Wolfgang Hampel, Berlin, FRG) with a solution containing the acetoxymethyl derivative of BCECF (18, 21, 22). The luminal perfusion fluid also contained FD and C green dye, which allowed delineation of more distal loops of the same nephron. This pipette will subsequently be referred to as the loading pipette. After 5–7 min, the loading pipette was removed and a second luminal pipette was placed in a more distal loop of the same nephron. This pipette was similar to that used in the peritubular capillary except that it had a smaller tip (7–9 μm). We have previously demonstrated that this technique allows control of luminal and peritubular fluid composition, and allows rapid changes in composition (18, 19).

The perfusion solutions are listed in Table I. All solutions were gassed with 7% CO_2/93% O_2. 4-Acetamido-4'-isothiocyano-(2,2')-disulfonic-stilbene (SITS) was obtained from International Chemical and Nuclear, Cleveland, OH, and (4,4')-diisothiocyano-(2,2')-disulfonic-stilbene (DIDS) from Sigma Chemical Co., St. Louis, MO. Ionized calcium concentration was measured on all perfusates (Nova 8, Nova Biomedical, Newton, MA) and was 1.3–1.5 mM. The luminal loading perfusion pipettes were filled with the control luminal solution (luminal perfusate used in the control period) containing 0.025% FD + C green dye No. 3 and 60 μg/ml (7.5×10^{-5} M) of the acetoxymethyl derivative of BCECF

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1. *Abbreviations used in this paper:* BCECF, (2',7')-bis(carboxyethyl)-(5,6)-carboxyfluorescein; BCECF-AM, acetoxymethyl derivative of BCECF; DIDS, (4,4')-diisothiocyano-(2,2')-disulfonic-stilbene; MPD, mean paired difference; PCT, proximal convoluted tubule; PST, proximal straight tubule; SITS, 4-acetamido-4'-isothiocyano-(2,2')-disulfonic-stilbene.

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Table I. Perfusion Solutions

	$25HCO_3$	$25HCO_3/0Cl$	$5HCO_3$	$5HCO_3/0Cl$	$25HCO_3$/Form	$25HCO_3$/Form/0Cl	$25HCO_3$/Acet	$25HCO_3$/Acet/0Cl
Na^+	147	147	147	147	147	147	147	147
K^+	5	5	5	5	5	5	5	5
Mg^{++}	1	1	1	1	1	1	1	1
Ca^{++}	1.8	9.3	1.5	9.3	1.8	9.3	1.8	9.3
Cl^-	128.6	—	148	—	127.6	—	127.6	—
Gluconate$^-$	—	143.6	—	163.6	—	142.6	—	142.6
HCO_3^-	25	25	5	5	25	25	25	25
$HPO_4^=$	1	1	1	1	1	1	1	1
$SO_4^=$	1	1	1	1	1	1	1	1
Formate$^-$	—	—	—	—	1	1	—	—
Acetate$^-$	—	—	—	—	—	—	1	1
Glucose	5	5	5	5	5	5	5	5
Alanine	5	5	5	5	5	5	5	5
Urea	5	5	5	5	5	5	5	5

(BCECF-AM) (Molecular Probes, Inc., Junction City, OR). This compound does not fluoresce and is lipid soluble. It rapidly diffuses into cells where cytoplasmic esterases cleave off the acetoxymethyl groups forming the fluorescent BCECF, which has four to five negative charges and thus leaves the cell slowly (23). Cells were usually loaded until sufficient visible fluorescence was achieved. We have previously demonstrated that tubules perfused with BCECF-AM have normal rates of volume and bicarbonate transport (18).

Cell pH measurement. After placement of the pipettes, the dissecting microscope was moved out of position, and a Leitz epifluorescence microscope (MPV Compact system, E. Leitz Inc.) was moved into position. Cell pH was then measured as previously described (18). Using an adjustable measuring diaphragm, fluorescence was measured in the loop that had contained the loading pipette, distal to the hole left by removal of the loading pipette, and never through the glass pipettes. Measurements were made using a 10X objective. The measured loop was always well within the capillary perfusion area. Background fluorescence was measured in a tubule that did not contain the dye but was within the area of capillary perfusion. Background varied only slightly from tubule to tubule.

Analysis. As described previously (18), epifluorescent emission was measured at 530 nm during alternate excitation at 500 (pH-sensitive wavelength) and 450 nm (pH-insensitive wavelength), accomplished with interference filters (Corion Corp., Holliston, MA). Fluorescence was always measured with 500 nm excitation followed by 450 nm excitation, followed again by 500 nm excitation. All results were corrected by subtracting background. The fluorescence excitation ratio (F_{500}/F_{450}) was calculated as the mean of the two 500-nm excitation measurements divided by the 450-nm excitation measurement. Use of the fluorescence excitation ratio provides a measurement unaffected by changes in dye concentration.

To convert fluorescent excitation ratios to an apparent cell pH value, results of our previously reported intracellular calibration were used (18, 24). Comparisons within the same tubule were made using the paired *t* test. Group comparisons not within the same tubule were made using covariance analysis. Results are reported as mean±SE.

Results

To establish if Cl/HCO_3 exchange was present on the apical membrane, the effect of adding or removing luminal chloride was examined. Entry of chloride into the lumen from the peritubular interstitium was prevented by perfusing peritubular capillaries in all studies with chloride-free solutions. In the first three sets of studies, lumens were perfused with chloride-free solutions in the control and recovery periods and with chloride-containing solutions in the experimental period, whereas capillaries were perfused with the same chloride-free solution in all three periods.

In the first set of studies, the lumen was perfused with a chloride-free solution containing 25 meq/liter bicarbonate (perfusate $25HCO_3/0Cl$, Table I) in the control and recovery periods. In the experimental period, luminal chloride concentration was increased to 128.6 meq/liter (perfusate $25HCO_3$, Table I). Capillaries were perfused with solution $25HCO_3/0Cl$ throughout. Cell pH was 7.29±0.03 in the control period, 7.31±0.04 in the experimental period, and 7.28±0.04 in the recovery period (all changes NS; Fig. 1, *solid circles*).

To examine if a lower cell pH is required for apical membrane Cl/HCO_3 exchange, similar studies were next performed with an extracellular bicarbonate concentration of 5 meq/liter. Lumen were perfused in the control and recovery periods with perfusate $5HCO_3/0Cl$ and with solution $5HCO_3$ ([Cl] = 148 meq/liter) in the experimental period (Table I). Capillaries were perfused with solution $5HCO_3/0Cl$ throughout. Once again, cell pH was unaffected by luminal chloride addition (7.00±0.03 in the control period, 6.99±0.05 in the experimental period, and 6.97±0.04 in the recovery period; Fig. 1, *open circles*).

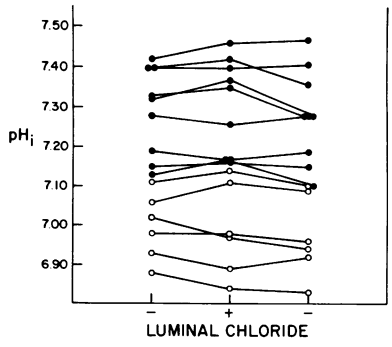

Figure 1. Effect of luminal chloride on cell pH. Lumen were perfused with solution $25HCO_3/0Cl$ (*closed circles*) or $5HCO_3/0Cl$ (*open circles*) in the control and recovery periods and with their respective chloride containing perfusate ($25HCO_3$ and $5HCO_3$) in the experimental period (Table I). Capillaries were perfused with the luminal control solution throughout.

To examine if a Cl/formate exchanger was present, the above studies were repeated with 1 mM formate present in luminal and peritubular fluids. Lumens were perfused with solutions $25HCO_3$/Form/0Cl in the control and recovery periods, and with solution $25HCO_3$/Form in the experimental period (Table I). Capillaries were perfused with solution $25HCO_3$/Form/0Cl throughout. Cell pH was 7.17±0.04 in the control period, 7.19±0.04 in the experimental period and 7.18±0.04 in the recovery period (all changes NS).

Thus, all studies reported thus far failed to demonstrate apical membrane Cl/base exchange. In previous studies, we found that basolateral membrane bicarbonate transport mechanisms were able to modify the response of cell pH to changes in apical membrane proton flux (19). As we (24) have recently found Cl/base exchange on the basolateral membrane, it is possible that increases in cell chloride concentration secondary to increases in luminal chloride concentration could drive basolateral membrane Cl/base exchange in a direction (bicarbonate influx) that would oppose apical membrane Cl/base exchange (bicarbonate efflux). In addition, basolateral membrane mechanisms could blunt any cell pH change (defend cell pH). Therefore we next attempted to demonstrate apical membrane Cl/base exchange after inhibition of basolateral membrane bicarbonate transport with peritubular SITS. This maneuver was previously used by us to demonstrate apical membrane Na/H exchange (19). In these studies (Figs. 2–5), peritubular capillaries were perfused with the control luminal solution with 1 mM SITS added.

In the first studies, lumens were perfused with chloride-free solutions in the control and recovery periods (perfusate $25HCO_3$/0Cl, Table I), and chloride was added in the experimental period (perfusate $25HCO_3$, Table I). In these studies, luminal chloride addition caused cell pH to decrease from 7.63±0.04 to 7.60±0.04 ($P < 0.005$; Fig. 2). When chloride was removed in the recovery period, cell pH returned to 7.63±0.04 ($P < 0.005$). Thus after inhibition of the basolateral membrane transport mechanisms with peritubular SITS, we were able to demonstrate Cl/base exchange (mean paired difference [MPD] = 0.02±0.01 pH U).

To examine if formate addition increased the magnitude of this apparent Cl/base exchange, the above studies were repeated with 1 mM formate added to all luminal and peritubular solutions (perfusates $25HCO_3$/Form/0Cl and $25HCO_3$/Form, Table I). Cell pH was 7.45±0.03 in the absence of luminal chloride in the control period, decreased to 7.38±0.02 upon luminal chloride addition, and returned to 7.44±0.02 when luminal chloride was removed in the recovery period (all changes $P < 0.001$; MPD = 0.06±0.01 pH U; Fig. 3). In the presence of formate, luminal chloride addition had a greater effect on cell pH (0.06 pH U) than in its absence (0.02 pH U; $P < 0.001$).

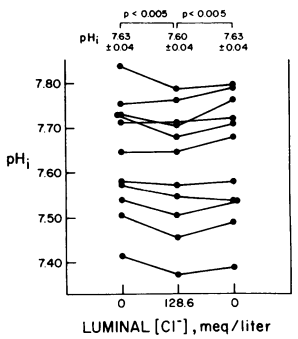

Figure 2. Effect of luminal chloride on cell pH after inhibition of basolateral membrane bicarbonate transport. Lumen were perfused with solution $25HCO_3$/0Cl in the control and recovery periods, and with solution $25HCO_3$ in the experimental period. Capillaries were perfused with solution $25HCO_3$/0Cl with 1 mM SITS added throughout.

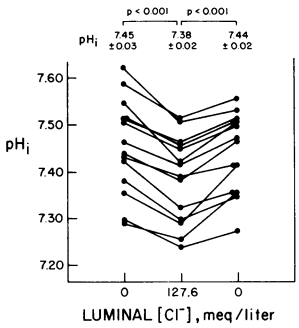

Figure 3. Effect of luminal chloride on cell pH after inhibition of basolateral membrane bicarbonate transport and in the presence of formate. Lumen were perfused with solution $25HCO_3$/Form/0Cl in the control and recovery periods, and with solution $25HCO_3$/Form in the experimental period. Capillaries were perfused with solution $25HCO_3$/Form/0Cl with 1 mM SITS added throughout.

To examine if this stimulation of Cl/base exchange was specific for formate, the above studies were repeated with 1 mM acetate added to all luminal and peritubular solutions (perfusates $25HCO_3$/Acet/0Cl and $25HCO_3$/Acet, Table I). As above, SITS was added to the peritubular fluid. Cell pH was 7.52±0.07 in the absence of luminal chloride in the control period, decreased to 7.50±0.07 upon luminal chloride addition ($P < 0.005$), and returned to 7.52±0.07 when luminal chloride was removed in the recovery period ($P < 0.01$; MPD = 0.03±0.01 pH U; Fig. 4). In the presence of acetate, luminal chloride addition had an effect on cell pH less than that seen in the presence of formate ($P < 0.001$) and similar to that seen in the absence of formate (NS).

In the next set of studies, the sensitivity of Cl/base exchange to luminal DIDS was examined. We chose to use luminal DIDS rather than SITS because Karniski and Aronson (16) and Schild et al. (25) used DIDS. For these studies, lumen and peritubular capillaries were perfused with perfusate $25HCO_3$/Form/0Cl in the control and recovery periods (Table I). In the experimental period, chloride was added to the luminal fluid (perfusate $25HCO_3$/Form). All luminal solutions contained 0.2 mM DIDS, whereas the peritubular perfusate contained 1 mM SITS. Cell pH was 7.46±0.01 in the control period, 7.47±0.02 in the experimental period, and 7.47±0.02 in the recovery period (all changes NS; Fig. 5). Thus, apical membrane Cl/base exchange is blocked by DIDS.

Discussion

Lucci and Warnock (8) first found that luminal SITS and high concentrations of furosemide inhibit NaCl absorption in the rat PCT. Subsequently, Baum (26) found a similar effect of SITS and DIDS in the rabbit PCT. As these compounds all inhibit the red blood cell Cl/HCO_3 exchanger (27, 28), these studies suggested that parallel Na/H and Cl/HCO_3 exchangers might

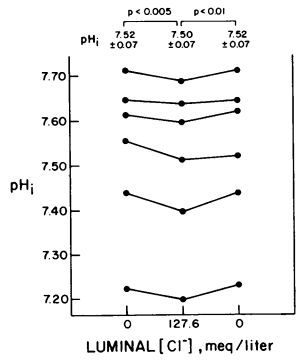

Figure 4. Effect of luminal chloride on cell pH after inhibition of basolateral membrane bicarbonate transport and in the presence of acetate. Lumen were perfused with solution $25HCO_3$/Acet/0Cl in the control and recovery periods, and with solution $25HCO_3$/Acet in the experimental period. Capillaries were perfused with solution $25HCO_3$/Acet/0Cl with 1 mM SITS added throughout.

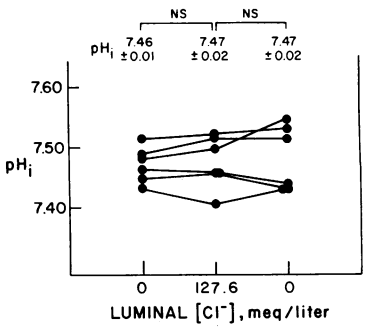

Figure 5. DIDS inhibition of apical membrane Cl/base exchange. Protocol as in Fig. 3, except that 0.2 mM DIDS was present in all luminal perfusates.

effect apical membrane NaCl absorption. Baum (26) also found that luminal DIDS stimulated net bicarbonate absorption, consistent with Cl/HCO_3 exchange.

Studies in isolated brush border (apical) membranes have conflicted with respect to the mechanism of chloride transport. Whereas all of these vesicle studies have found a large chloride conductance, some have found Cl/OH exchange (9–12), and some have not (13–15). Karniski and Aronson (16) demonstrated the presence of DIDS-sensitive Cl/formate exchange in apical membranes. These authors postulated that if the apical membrane was highly permeable to formic acid, then a Cl/formate exchanger would be equivalent to a Cl/OH exchanger and, in parallel with Na/H exchange, could affect apical membrane NaCl transport. In support of this thesis, Schild et al. (25) recently found that addition of small concentrations of formate to the luminal and bath fluid stimulated NaCl absorption in rabbit PCT and proximal straight tubule (PST) perfused in vitro. This effect was blocked by DIDS.

Studies that have attempted to demonstrate directly apical membrane anion exchange in intact tubules generally have been negative. Schwartz (29) measured the rate of luminal alkalinization in rabbit PCT perfused with acid solutions and was unable to find an effect of luminal chloride. Sasaki et al. (30) found no effect of luminal chloride removal on cell pH in the rabbit PST.

In the present studies, no effect of luminal chloride on cell pH was found in the presence of intact basolateral membrane transport mechanisms. These studies demonstrate that apical membrane anion exchangers are not important determinants of cell pH. In addition, these studies do not support the presence of a significant apical membrane chloride conductance in vivo. As these cells possess a rheogenic potential difference-sensitive bicarbonate transport mechanism (18, 31–36), changes in cell potential difference would have been expected to cause changes in cell pH.

In previous studies (19), we found that SITS-sensitive basolateral membrane transport mechanisms were able to modify the cell pH response to changes in luminal sodium and pH. Therefore we repeated the present studies in the presence of peritubular SITS. In this setting, luminal chloride addition caused a significant 0.02 pH U cell acidification that increased to 0.06 pH U in the presence of formate but was only 0.03 pH U in the presence of acetate (Fig. 6). The presence of Cl/base exchange in the absence of formate suggests that some Cl/OH,HCO_3 exchange is present. As peritubular SITS inhibits the cell's bicarbonate conductance (18, 31–36), these results cannot be explained by an apical membrane chloride conductance. The enhancement of Cl/base exchange by formate supports the presence of Cl/formate exchange as found by Karniski and Aronson (16). Karniski and Aronson (16) also found that acetate could not substitute for formate on this transporter, in agreement with the present results.

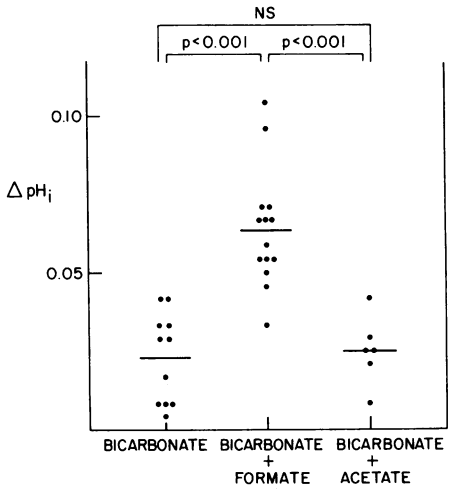

Figure 6. Comparison of the magnitudes of Cl/base exchange. The magnitude of the change in cell pH seen in each tubule upon luminal chloride addition and removal (average of the two pH changes) is plotted for each of the tubules in each of three protocols. In all studies, SITS was present in the peritubular capillary. The three protocols included studies performed in the presence of bicarbonate only (Fig. 2), studies with formate (Fig. 3), and those with acetate (Fig. 4).

These conclusions are consistent with the finding that active transcellular chloride absorption can be demonstrated in the in vitro perfused rabbit PCT in the absence of added formate (3, 25) but is accelerated by addition of formate (25). In the rat, studies that have demonstrated active chloride absorption have been performed with blood, possibly containing formate, in the capillaries (1, 4). The failure to consistently find Cl/OH exchange in vesicle studies may be due to the small magnitude of exchange compared to the large magnitude of the chloride conductance (in the vesicles). Alternatively, it is possible that in our studies and in the rabbit tubule studies performed in the absence of formate, metabolic production by the proximal tubular cell maintains sufficient levels of formate in the cell.

In summary, the above studies demonstrate apical membrane stilbene-sensitive Cl/base exchange. More than half of this appears to be Cl/formate exchange with the remaining fraction probably Cl/OH,HCO_3 exchange. These mechanisms, in parallel with Na/H antiport, provide a pathway for NaCl absorption across the apical membrane. The secreted H^+ and HCO_3^- or formate will neutralize each other in the lumen and then diffuse across the apical membrane as CO_2 and formic acid, respectively.

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