Basolateral Membrane Cl/HCO₃ Exchange in the Rat Proximal Convoluted Tubule

Na-dependent and -independent Modes

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ABSTRACT   To examine whether Cl-coupled HCO₃ transport mechanisms were present on the basolateral membrane of the mammalian proximal tubule, cell pH was measured in the microperfused rat proximal convoluted tubule using the pH-sensitive, intracellularly trapped fluorescent dye (2',7')-bis(carboxyethyl)-(5,6)-carboxyfluorescein. Increasing the peritubular Cl concentration from 0 to 128.6 meq/liter caused cell pH to decrease from 7.34 ± 0.04 to 7.21 ± 0.04 ($p < 0.001$). With more acid extracellular fluid (pH 6.62), a similar increase in the peritubular Cl concentration caused cell pH to decrease by a similar amount from 6.97 ± 0.04 to 6.84 ± 0.05 ($p < 0.001$). This effect was blocked by 1 mM SITS. To examine the Na dependence of Cl/HCO₃ exchange, the above studies were repeated in the absence of luminal and peritubular Na. In alkaline Na-free solutions, peritubular Cl addition caused cell pH to decrease from 7.57 ± 0.06 to 7.53 ± 0.06 ($p < 0.025$); in acid Na-free solutions, peritubular Cl addition caused cell pH to decrease from 7.21 ± 0.04 to 7.19 ± 0.04 ($p < 0.05$). The effect of Cl on cell pH was smaller in the absence of luminal and peritubular Na than in its presence. To examine whether the previously described $Na/(HCO_3)_{n>1}$ cotransporter was coupled to or dependent on Cl, the effect of lowering the peritubular Na concentration from 147 to 25 meq/liter was examined in the absence of ambient Cl. Cell pH decreased from 7.28 ± 0.03 to 7.08 ± 0.03, a response similar to that observed previously in the presence of Cl. The results demonstrate that Cl/HCO₃ (or Cl/OH) exchange is present on the basolateral membrane. Most of Cl/HCO₃ exchange is dependent on the presence of Na and may be coupled to it. The previously described $Na/(HCO_3)_{n>1}$ cotransporter is the major basolateral membrane pathway for the coupling of Na and HCO₃ and is not coupled to Cl.

INTRODUCTION

The possible existence of a basolateral membrane Cl/HCO₃ exchanger in the renal proximal tubule was first raised by studies in the *Necturus* proximal tubule,

in which Edelman et al. (1981) found that HCO_3 gradients altered the intracellular Cl concentration in a manner consistent with Cl/HCO_3 exchange. Subsequent studies by Guggino et al. (1983) confirmed this finding, but suggested that the transporter was also coupled to Na in such a way that it functioned as an $NaHCO_3/Cl$ exchanger. In the mammalian proximal tubule, studies on isolated basolateral membranes have found anion exchangers capable of exchanging Cl for HCO_3 (Low et al., 1984; Grassal et al., 1986).

The purpose of the present studies was to examine whether Cl/HCO_3 exchange is present on the basolateral membrane of the mammalian proximal convoluted tubule (PCT). To accomplish this, we examined the effect of Cl gradients across the basolateral membrane on cell pH. Cell pH was measured in the in vivo microperfused rat PCT using the pH-sensitive, intracellularly trapped fluorescent probe (2',7')-bis(carboxyethyl)-(5,6)-carboxyfluorescein (BCECF). The results demonstrate basolateral membrane Cl/HCO_3 exchange, most of which is dependent on the presence of Na and is possibly coupled to it.

METHODS

Experiments were performed using male Wistar rats (Charles River Breeding Laboratories, Inc., Wilmington, MA) weighing 180–303 g. The rats were prepared for microperfusion as previously described (Alpern, 1984). 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 right femoral artery was catheterized for monitoring blood pressure and obtaining blood samples. The left kidney was exposed using a flank incision and immobilized in a Lucite cup. The ureter was cannulated (PE-50) to ensure the free drainage of urine. Rats were infused intravenously with an HCO_3 Ringer's solution (105 mM NaCl, 25 mM $NaHCO_3$, 4 mM Na_2HPO_4, 5 mM KCl, 1 mM $MgSO_4$, and 1.8 mM $CaCl_2$) at 3.2 ml/h during surgery, and then at 1.6 ml/h throughout the rest of the experiment. The proximal tubular transit time was measured after injection of 0.02 ml of 10% lissamine green dye intravenously, and only kidneys in which the transit time was <11 s were accepted for study. At the completion of surgery, a blood sample was obtained for determination of pH and P_{CO_2} (model 165 blood gas analyzer, Corning Glass Works, Medfield, MA). The rats had normal systemic acid-base parameters: pH = 7.44 ± 0.04, P_{CO_2} = 38.1 ± 0.6 mmHg, and $[HCO_3^-]$ = 24.5 ± 1.7 mM.

Pipettes were placed using a Leitz dissecting microscope (Leitz Wetzlar, Rockleigh, NJ). Peritubular capillaries were perfused as previously described (Alpern, 1985; Alpern and Chambers, 1986) 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 (Alpern and Chambers, 1986) using a technique of rapid retrograde perfusion similar to that described by Frömter and Gessner (1974). First, tubules were perfused at 40 nl/min for 5–7 min using a thermally insulated microperfusion pump (Wolfgang Hampel, Berlin, Federal Republic of Germany) with a solution containing the acetoxymethyl derivative of BCECF (see below), as previously described (J. A. Thomas et al., 1979; Rink et al., 1982; Moolenaar et al., 1983; Alpern, 1985). The luminal perfusion fluid also contained FD + C green dye, which allowed delineation of more distal loops of the same nephron. This luminal 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 (see above), except that it had a smaller tip (7–9 μm). We have previously demonstrated

(Alpern, 1985; Alpern and Chambers, 1986) that this technique allows control of the luminal and peritubular fluid composition and allows rapid changes in composition.

The perfusion solutions are listed in Table I. All solutions were gassed with 7% CO_2/ 93% O_2. SITS (4-acetamido-4'-isothiocyanostilbene-2,2'-disulfonate) was obtained from Sigma Chemical Co. (St. Louis, MO). The ionized Ca concentration was measured in all perfusates (Nova 8, Nova Biomedical, Newton, MA) and adjusted to 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 (BCECF-AM) (Research Development Corp., Toronto, Ontario, and 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

TABLE I
Compositions of Solutions

	25 HCO₃/ 128.6 Cl	25 HCO₃/ 0 Cl	5 HCO₃/ 148 Cl	5 HCO₃/ 0 Cl	25 HCO₃/ 0 Na/ 128.6 Cl	25 HCO₃/0 Na/0 Cl	5 HCO₃/0 Na/148 Cl	5 HCO₃/0 Na/0 Cl	25 HCO₃/ 25 Na/0 Cl
Na⁺	147	147	147	147	—	—	—	—	25
K⁺	5	5	5	5	5	5	5	5	5
Mg²⁺	1	1	1	1	1	1	1	1	1
Ca²⁺	1.8	9.3	1.5	9.3	1.8	9.3	1.5	9.3	9.3
Choline⁺	—	—	—	—	147	147	147	147	122
Cl⁻	128.6	—	148	—	128.6	—	148	—	—
Gluconate⁻	—	143.6	—	163.6	—	143.6	—	163.6	143.6
HCO₃⁻	25	25	5	5	25	25	5	5	25
HPO₄	1	1	1	1	1	1	1	1	1
SO₄	1	1	1	1	1	1	1	1	1
Glucose	5	5	5	5	5	5	5	5	5
Alanine	5	5	5	5	5	5	5	5	5
Urea	5	5	5	5	5	5	5	5	5
pH	7.32	7.32	6.63	6.63	7.32	7.32	6.63	6.63	7.32

All units are millimolar.

BCECF, which has four negative charges and thus leaves the cell slowly. Cells were usually loaded until sufficient visible fluorescence was achieved. In previous studies (Alpern, 1985), the half-time for loss of BCECF from the cell was found to be 10–12 min at 37°C in the rat PCT.

We have previously demonstrated (Alpern, 1985) that tubules perfused with BCECF-AM have normal rates of volume and HCO₃ transport. Thus, the dye itself is not cytotoxic. It can, however, cause photodynamic damage to the cell. Indeed, if prolonged light exposure occurs, cells swell and cell pH decreases. When this was noted, the data were rejected. To avoid this problem, minimal light exposure was used.

Cell pH Measurements

After placement of the pipettes, the dissecting microscope was moved out of position and a Leitz epifluorescence microscope (MPV compact system, Leitz Wetzlar) was moved into position. Cell pH was then measured as previously described (Alpern, 1985). In general,

the fluorescence intensity was greatest in the cells of the loop in which the loading pipette was located (distal to the location of the loading pipette) and was smaller in subsequent loops. The lumens, capillaries, and surrounding structures did not fluoresce above background. 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. This was done by use of an adjustable measuring diaphragm. Measurements were made using a 10× objective. Although the size of the measured area varied, it was usually ~60–80 μm square. 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. The background varied only slightly from tubule to tubule.

Analysis

BCECF has a peak excitation at 504 nm that is pH sensitive, and an isosbestic point (where fluorescence excitation is independent of pH) at 436 nm (Alpern, 1985). The peak emission is at 526 nm. As described previously (Alpern, 1985), epifluorescent emission was measured at 530 nm during alternate excitation at 500 and 450 nm, which was accomplished with interference filters (Corion Corp., Holliston, MA). Fluorescence was always measured with 500 nm excitation, followed by 450 nm excitation, and followed again by 500 nm excitation. All results were corrected by subtracting the 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 that is unaffected by changes in dye concentration.

In order to convert fluorescent excitation ratios to an apparent cell pH value, the results of our previously reported intracellular calibrations were used for BCECF-AM acquired from Research Development Corp. (Alpern, 1985). To determine whether the BCECF-AM acquired from Molecular Probes was similar, we repeated these studies. Tubules were perfused as previously described (Alpern, 1985), with well-buffered solutions (25 mM HEPES, 60 mM phosphate, and appropriate HCO_3 concentrations) containing 10 μg/cc (1.34×10^{-5} M) nigericin (a K/H antiporter) and 120 meq/liter K (estimated to approximate cell K activity [Alpern, 1985]) at various pH values. Surprisingly, the results demonstrated that the BCECF-AM from Molecular Probes (filled squares, Fig. 1) forms a slightly different compound intracellularly than that formed by the BCECF-AM acquired from Research Development Corp. (filled circles, Fig. 1). Although the slopes of a plot of excitation ratio vs. pH were not different, the Y-intercepts were shifted (Fig. 1). Using the results of each dye with its respective calibration gave similar calculated cell pH values, confirming the value of the nigericin calibration. It should also be noted that whereas the nigericin calibration using BCECF-AM from Research Development Corp. was shifted up 0.6 pH unit from the in vitro calibration of BCECF (open symbols, Fig. 1), the nigericin calibration using the BCECF-AM from Molecular Probes was only shifted ~0.2 pH unit. At present, there is no explanation for this discrepancy.

Comparisons within the same tubule were made using the paired t test. Group comparisons not within the same tubule were made using the unpaired t test. Results are reported as means ± SE. All studies were performed in tubules from at least two animals.

RESULTS

Cl/HCO₃ Exchange

The first set of studies was designed to determine whether gradients could drive an HCO_3 flux across the basolateral membrane. In the control and recovery

periods, the lumen and peritubular capillaries were perfused with a solution containing no Cl and 25 meq/liter HCO$_3$ (perfusate 25 HCO$_3$/0 Cl, pH 7.32, Table I). In the experimental period, peritubular Cl was increased to 128.6 meq/liter (perfusate 25 HCO$_3$/128.6 Cl, pH 7.32, Table I). Fig. 2 shows a tracing from a typical study. The intensity of fluorescence is indicated by the height of the bars. Measurements with 450 nm excitation are indicated by triangles over
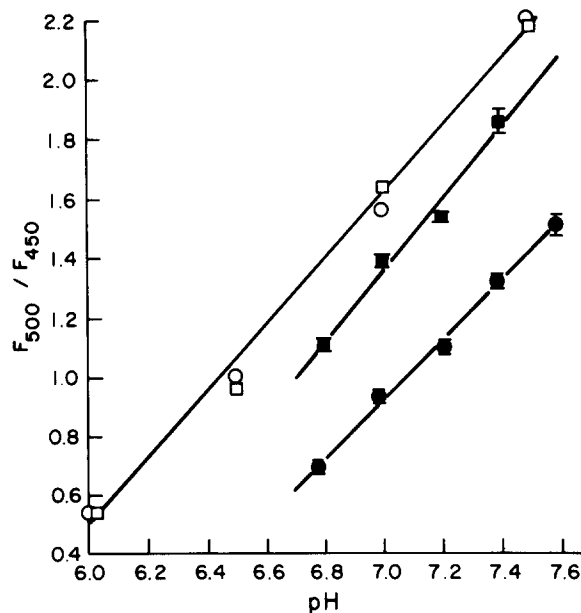

FIGURE 1. Dye calibration by epifluorescent microscopy. The open circles represent the previously reported in vitro calibration of BCECF from Research Development Corp. (Alpern, 1985). The open squares represent the in vitro calibration of BCECF from Molecular Probes, Inc. This calibration was performed using a potassium phosphate buffer with 10 μM BCECF as previously described (Alpern, 1985). The filled circles represent the previously reported intracellular calibration of BCECF obtained from Research Development Corp. (Alpern, 1985). The filled squares represent the intracellular calibration of BCECF obtained from Molecular Probes. The compounds generated within the cells are similar in that the slopes of the fluorescent excitation ratio vs. pH are similar. The intercepts, however, are markedly different. Whereas the apparent pK of the Research Development Corp. dye intracellularly is shifted 0.6 pH unit from the compound in vitro, the Molecular Probes compound generated intracellularly is shifted only 0.2 pH unit from the in vitro calibration.

the bars, while all other measurements were with 500 nm excitation. When Cl was added to the peritubular capillaries, there was an immediate cell acidification, which was indicated by an immediate decrease in fluorescence measured with 500 nm excitation and a decrease in the ratio of 500–450 nm fluorescence. There was no recovery of cell pH over 1–2 min while Cl was present. Removal of peritubular Cl reversed these effects. In similar studies on 14 tubules, cell pH

was 7.34 ± 0.04 in the control period, decreased to 7.21 ± 0.04 in the experimental period, and returned to 7.36 ± 0.03 in the recovery period ($p <$ 0.001, control vs. experimental period; $p < 0.001$, experimental vs. recovery period; Fig. 3).

Similar results were found when studies similar to those described above were performed with a lower luminal and peritubular pH. In the control and recovery periods, the lumen and peritubular capillaries were perfused with a solution containing no Cl and 5 meq/liter HCO_3 (perfusate 5 HCO_3/0 Cl, pH 6.63, Table I). In the experimental period, peritubular Cl was increased to 148 meq/liter (perfusate 5 HCO_3/148 Cl, pH 6.63, Table I). Cell pH was 6.97 ± 0.04 in the
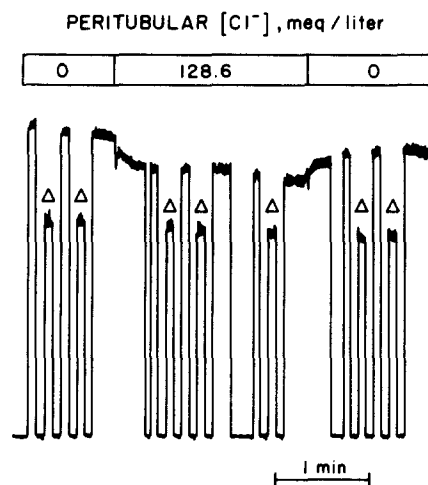
PERITUBULAR $[Cl^-]$, meq / liter

FIGURE 2.   Effect of peritubular Cl concentration on cell pH; results of a typical study are shown. The lumen and capillaries were perfused with Cl-free solutions containing 25 meq/liter HCO_3. At the indicated time, the peritubular Cl concentration was increased to 128.6 meq/liter. The intensity of fluorescence in arbitrary units is indicated by the height of the bars. Measurements with 450 nm excitation are indicated by triangles over the bars. All other measurements were with 500 nm excitation. When Cl is added to the peritubular capillary fluid, a rapid decrease in 500 nm fluorescence occurs that is accompanied by a decrease in the ratio of 500 to 450 nm fluorescence. All changes reverse when Cl is removed from the capillary.

control period, decreased to 6.84 ± 0.05 in the experimental period, and returned to 6.97 ± 0.03 in the recovery period ($p < 0.001$, control vs. experimental period; $p < 0.005$, experimental vs. recovery period; Fig. 4). Thus, the effect of the peritubular Cl concentration on cell pH is of similar magnitude under acid and alkaline conditions.

SITS Inhibition

SITS inhibits anion exchangers in many tissues. To test whether basolateral membrane Cl/HCO_3 exchange was sensitive to SITS, studies were performed in the presence of 1 mM peritubular SITS. In the control and recovery periods,
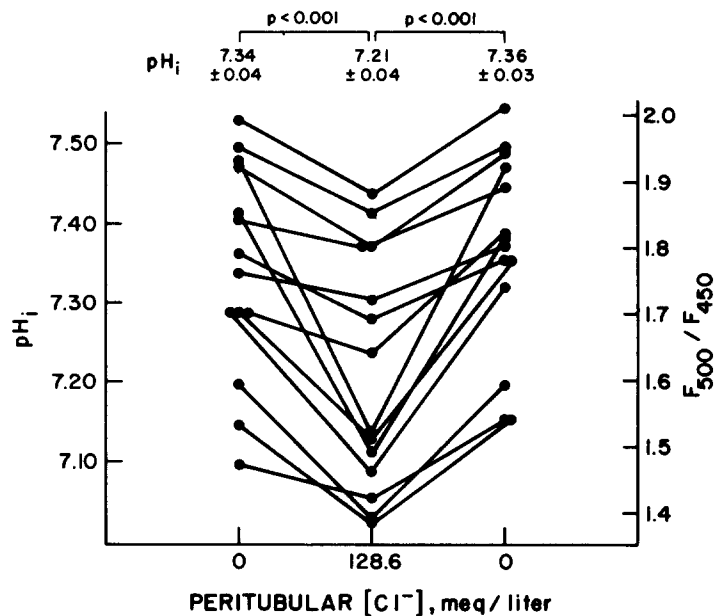
FIGURE 3.   Effect of peritubular Cl concentration on cell pH. The protocol was the same as that in Fig. 2. The ordinate shows the fluorescence excitation ratio on the right and the calculated cell pH on the left.
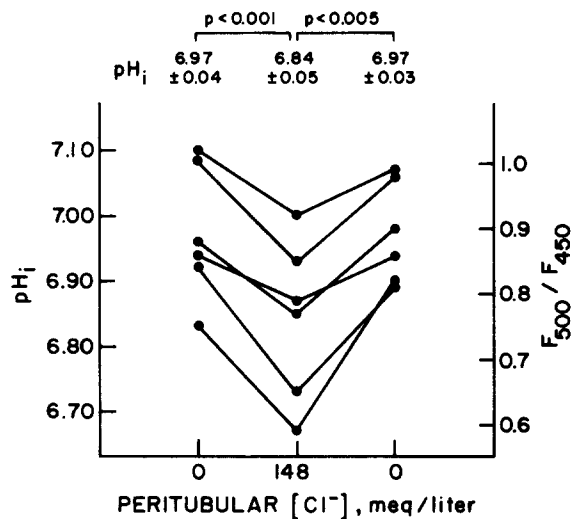

FIGURE 4.   Effect of peritubular Cl concentration on cell pH. The lumen and capillaries were perfused with Cl-free solutions containing 5 meq/liter HCO_3 during control and recovery periods. During the experimental period, the capillary Cl concentration was increased to 148 meq/liter. The ordinate shows the fluorescence excitation ratio on the right and the calculated cell pH on the left.

the lumen and peritubular capillaries were perfused with solution 25 $HCO_3/0$ Cl (Table I), while in the experimental period, peritubular capillaries were perfused with solution 25 $HCO_3/128.6$ Cl (Table I). All peritubular solutions contained 1 mM SITS. Cell pH was 7.37 ± 0.02 in the control period, 7.37 ± 0.02 in the experimental period, and 7.35 ± 0.02 in the recovery period (all changes NS; Fig. 5). Thus, the Cl/HCO_3 exchanger is inhibited by disulfonic stilbenes.

Dependence on Na

Guggino et al. (1983) found that the basolateral membrane Cl/HCO_3 exchanger of *Necturus* proximal tubule is coupled to Na. Therefore, we examined whether the effect of peritubular Cl on cell pH was dependent on the presence of Na. In
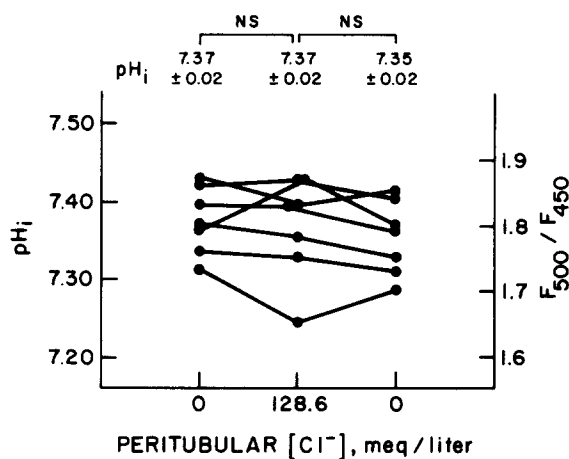

FIGURE 5. SITS inhibition of basolateral membrane Cl/HCO_3 exchange. The lumen and capillaries were perfused with Cl-free solutions containing 25 meq/liter HCO_3 during the control and recovery periods. During the experimental period, the capillary Cl concentration was increased to 128.6 meq/liter. All peritubular capillary solutions contained 1 mM SITS. The ordinate shows the fluorescence excitation ratio on the right and the calculated cell pH on the left.

the control and recovery periods, the lumen and capillaries were perfused with a solution containing no Na or Cl and 25 meq/liter HCO_3 (perfusate 25 $HCO_3/$ 0 Na/0 Cl, Table I), while in the experimental period, Cl was added to the peritubular capillaries (perfusate 25 $HCO_3/0$ Na/128.6 Cl, Table I). A tracing from a typical study is shown in Fig. 6. While small changes in the fluorescence excitation ratio occurred, they were clearly smaller than those seen in the presence of Na.

In similar studies on five tubules, cell pH was 7.57 ± 0.06 in the control period, 7.53 ± 0.06 in the experimental period, and 7.58 ± 0.06 in the recovery period ($p < 0.025$, control vs. experimental period; $p < 0.01$, experimental vs. recovery period; Fig. 7). In these studies, the addition of Cl caused cell pH to

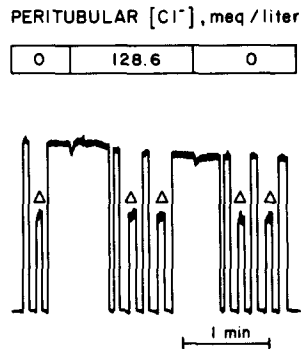PERITUBULAR [Cl⁻], meq / liter

O	128.6	O

FIGURE 6. Effect of peritubular Cl on cell pH in the absence of luminal and peritubular Na; results of typical study are shown. The lumen and capillaries were perfused with a solution containing no Na or Cl and 25 meq/liter HCO_3. At the indicated time, the peritubular Cl concentration was increased to 128.6 meq/liter. The intensity of fluorescence in arbitrary units is indicated by the height of the bars. Measurements with 450 nm excitation are indicated by triangles over the bars. All other measurements were with 500 nm excitation.

decrease, but the magnitude of the decrease was smaller than that observed in the presence of Na ($p < 0.05$).

In these studies, Na removal caused cell alkalinization, in agreement with our previous results (Alpern, 1985) and those of Nakhoul and Boron (1985b). This cell alkalinization may be due to the fact that after inhibition of Na-coupled
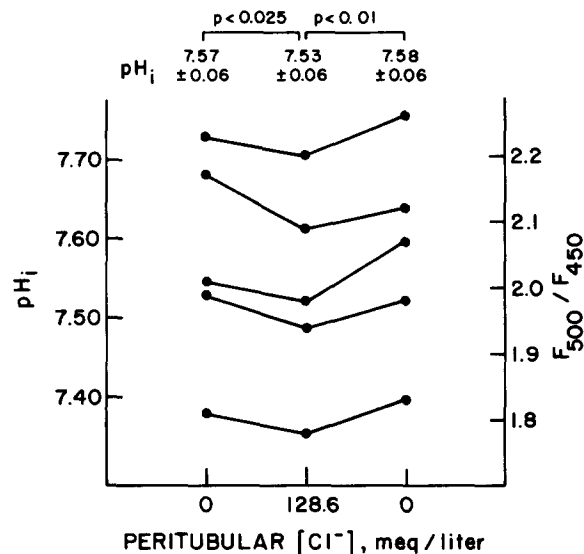

PERITUBULAR [Cl⁻], meq / liter

FIGURE 7. Effect of peritubular Cl on cell pH in the absence of luminal and peritubular Na. The protocol was the same as in Fig. 6. The ordinate shows the fluorescence excitation ratio on the right and the calculated cell pH on the left.

H^+/HCO_3^- transporters on both membranes, the apical membrane H^+-ATPase becomes a more dominant determinant of cell pH.

Because it was possible that the high cell pH could have caused the decrease in ΔpH, the above studies were repeated with more acid luminal and peritubular fluids. In the control and recovery periods, the lumen and peritubular capillaries were perfused with a solution containing no Na or Cl and 5 meq/liter HCO_3 (perfusate 5 HCO_3/0 Na/0 Cl, Table I), while in the experimental period, the capillaries were perfused with a similar solution containing Cl (perfusate 5 HCO_3/ 0 Na/148 Cl, Table I). Cell pH was 7.21 ± 0.04 in the control period, 7.19 ± 0.04 in the experimental period, and 7.21 ± 0.04 in the recovery period ($p <$ 0.05, control vs. experimental period; $p < 0.05$, experimental vs. recovery period; Fig. 8). Once again, the magnitude of the cell pH change was smaller than that
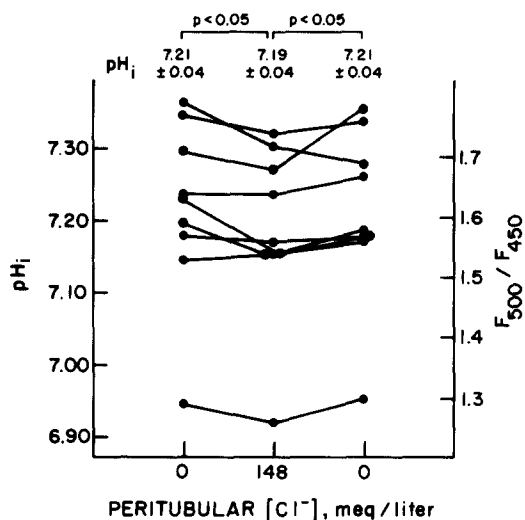

FIGURE 8.   Effect of peritubular Cl on cell pH in the absence of luminal and peritubular Na. The lumen and capillaries were perfused with a solution containing no Na or Cl and 5 meq/liter HCO_3 during the control and recovery periods. During the experimental period, the capillary Cl concentration was increased to 148 meq/ liter. The ordinate shows the fluorescence excitation ratio on the right and the calculated cell pH on the left.

observed in the presence of Na ($p < 0.001$). Thus, while a small amount of Cl/ HCO_3 exchange persists in the absence of Na, it is of much greater magnitude in the presence of Na, which suggests that most of Cl/HCO_3 exchange is dependent on the presence of Na and is possibly coupled to it.

To gain further insight into the mechanism of this Na dependence, we examined the effect of separately removing luminal or peritubular Na on basolateral membrane Cl/HCO_3 exchange. First, Na was removed from the capillary. The lumens were perfused with a solution containing Na but no Cl (perfusate 25 HCO_3/0 Cl, Table I), and the capillaries were perfused with a solution containing no Na or Cl (perfusate 25 HCO_3/0 Na/0 Cl, Table I) in the control and recovery periods and with a similar solution containing Cl (perfusate

25 HCO$_3$/0 Na/128.6 Cl, Table I) in the experimental period. Cell pH was 7.20 ± 0.02 in the control period, 7.13 ± 0.03 in the experimental period, and 7.18 ± 0.02 in the recovery period ($p < 0.01$, control vs. experimental period; $p < 0.05$, experimental vs. recovery period; $n = 6$).

Next, Na was removed from the lumen. The lumens were perfused with an Na-free, Cl-free perfusate (perfusate 25 HCO$_3$/0 Na/0 Cl, Table I), and the capillaries were perfused with a solution containing Na but no Cl (perfusate 25 HCO$_3$/0 Cl, Table I) in the control and recovery periods, and with a similar solution containing Cl in the experimental period (perfusate 25 HCO$_3$/128.6 Cl,
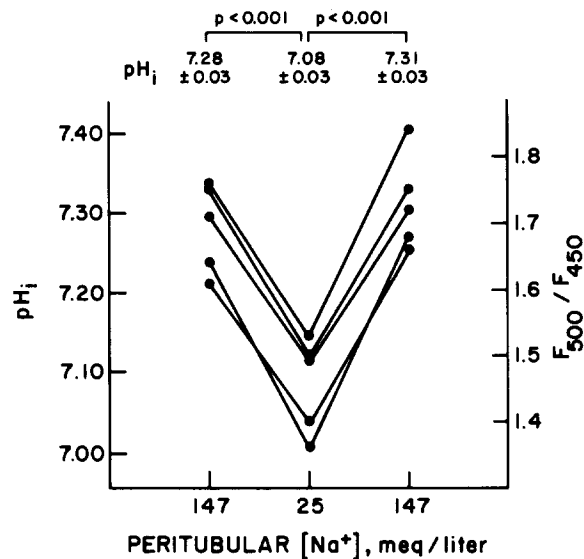

FIGURE 9. Effect of Cl removal on Na/HCO$_3$ cotransport. The lumen and peritubular capillaries were perfused with a solution containing no Cl, 147 meq/liter Na, and 25 meq/liter HCO$_3$ during the control and recovery periods. During the experimental period, the Na concentration in the capillary was lowered to 25 meq/liter. The ordinate shows the fluorescence excitation ratio on the right and the calculated cell pH on the left.

Table I). Cell pH was 7.65 ± 0.03 in the control period, 7.64 ± 0.04 in the experimental period, and 7.71 ± 0.05 in the recovery period (NS, control vs. experimental period; $p < 0.001$, experimental vs. recovery period; $n = 5$). In these studies, there was a steady alkalinization of the cell, which we have previously reported with luminal Na removal (Alpern and Chambers, 1986). This alkalinization, which also explains the very high cell pH values seen in these studies, is probably due to reversal of the basolateral membrane Na/(HCO$_3$)$_3$ symporter and to the functioning of an apical membrane H$^+$-ATPase. This steady alkalinization is the reason that the initial cell acidification upon addition of Cl is not significant. If the cell pH in the experimental period is compared with the average of the control and recovery periods, the difference, 0.04 ± 0.01 pH unit, is highly significant ($p < 0.005$).

The cell pH response both in the absence of luminal Na ($\Delta pH = 0.04 \pm 0.01$) and in the absence of capillary Na ($\Delta pH = 0.07 \pm 0.02$) is significantly lower than that seen in the presence of Na on both sides of the cells ($p < 0.05$ for both comparisons). Thus, Na removal from the lumen and capillary, or from the lumen or capillary separately, inhibits Cl/HCO_3 exchange. This suggests that the dependence on Na is on the cell Na concentration. The implications of this are considered in the Discussion.

We and others (Boron and Boulpaep, 1983; Alpern, 1985; Yoshitomi et al., 1985; Sasaki et al., 1986) have previously found that decreasing the peritubular Na concentration caused cell pH to decrease, an effect that was attributed to a Cl-independent Na/HCO_3 cotransporter. To examine further whether this pH change was due to an effect on a Cl-coupled transporter, we repeated these studies in the absence of Cl. In the control and recovery periods, the lumen and capillaries were perfused with a solution containing no Cl, 147 meq/liter Na, and 25 meq/liter HCO_3 (perfusate 25 HCO_3/0 Cl, Table I). In the experimental period, the peritubular Na concentration was decreased to 25 meq/liter (perfusate 25 HCO_3/25 Na/0 Cl, Table I). Cell pH decreased from 7.28 ± 0.03 in the control period to 7.08 ± 0.03 in the experimental period, and returned to 7.31 ± 0.03 in the recovery period ($p < 0.001$, control vs. experimental period; $p < 0.001$, experimental vs. recovery period; Fig. 9). This change in cell pH was not different from that previously observed by us in the presence of Cl (0.2 pH unit; Alpern, 1985). Thus, the major pathway for basolateral membrane Na/HCO_3 cotransport is independent of Cl.

DISCUSSION

In previous studies (Alpern, 1985), we found no effect on cell pH when peritubular Cl was lowered in the presence of luminal Cl. Because of the possibility that we had been unable to lower the interstitial Cl concentration sufficiently, we repeated these studies with Cl removed from the luminal fluid. The sudden addition of Cl to the peritubular fluid then caused cells to acidify by 0.10–0.15 pH units. This occurred both in acid cells perfused with acid luminal and peritubular solutions, and in more alkaline cells perfused with more alkaline luminal and peritubular fluids. Thus, the present studies demonstrate that Cl concentration gradients across the basolateral membrane cause the countertransport of HCO_3 or a base equivalent. In addition, this Cl/HCO_3 or Cl/OH exchange was sensitive to SITS.

In view of the previous demonstration of an electrogenic, voltage-sensitive mechanism for HCO_3 transport in the basolateral membrane of the proximal tubule (Boron and Boulpaep, 1983; Alpern, 1985; Biagi, 1985; Yoshitomi et al., 1985; Grassl and Aronson, 1986; Sasaki et al., 1986), the present effect of Cl on cell pH could have been due to an effect on the cell potential difference (PD). Such an effect on the cell PD would be anticipated if the basolateral membrane possessed a Cl conductance. The addition of peritubular Cl would then hyperpolarize the cell, which would drive HCO_3 out of the cell via the electrogenic basolateral membrane HCO_3 transporter. However, a number of studies have

failed to find a basolateral membrane Cl conductance in the rat proximal tubule. Burckhardt et al. (1984) found that total replacement of peritubular Cl with isethionate or sulfate did not affect the cell PD. Cassola et al. (1983) reported similar findings with gluconate substitution. Burckhardt et al. (1984) also reported that removal of Cl from the peritubular capillary did not affect the measured resistance of the basolateral membrane.

Similar conclusions have been drawn for the salamander (*Necturus*) proximal tubule. Shindo and Spring (1981) altered the cell potential by luminal current injection and did not find that the cell Cl concentration was affected in the direction predicted by the existence of a Cl conductance. Guggino et al. (1982) found that a reduction of the Cl concentration in the luminal and peritubular solutions did not affect the resistances of the apical and basolateral membranes. In addition, these authors found that lowering the Cl concentration in the luminal and peritubular fluid or in the peritubular fluid alone did not depolarize the cell. In summary, these studies suggest that neither the salamander nor the mammalian proximal tubule possesses a basolateral membrane Cl conductance.

Another possible mechanism for an electrically coupled effect of peritubular Cl concentration on cell pH is cell hyperpolarization secondary to circular currents. The replacement of peritubular gluconate with Cl will cause a lumen-negative transepithelial diffusion PD (caused by a Cl current across the paracellular pathway into the lumen), which will be associated with cell hyperpolarization (with respect to the peritubular compartment). The magnitude of the basolateral membrane hyperpolarization, however, will be only ~4 mV.[1] This calculation agrees with the studies described above, where cell PD was measured during changes in the peritubular Cl concentration and was found to be unaffected.

Another argument against circular currents as a cause of the cell pH change derives from the effect of luminal Cl addition. If circular currents are prominent, then luminal Cl addition should lead to a circular current of equal magnitude, but opposite in direction to that found with peritubular Cl addition. This should

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[1] To calculate the expected effect of transepithelial Cl gradients on the basolateral membrane voltage from circular current effects, the expected effect of the Cl gradient on the transepithelial PD was first calculated. Because the resistance of the paracellular pathway is much smaller than that of the cell (Frömter, 1982), it can be calculated using the Goldman equation with permselectivity properties of the paracellular pathway: $P_{Na}/P_{Cl} = 1.22$ and $P_{HCO}/P_{Cl} = 0.44$ (Frömter et al., 1971). For $P_{gluconate}/P_{Cl}$, a value of 0.25 was used that was based on values of 0.25 and 0.28 measured by Frömter et al. (1971) for sulfate and cyclamate. Using these values with the Goldman equation yields diffusion PD values of −9.2 and −10.5 mV in tubules perfused with 25 and 5 meq/liter bicarbonate, respectively. The effect of this transepithelial PD (V_{te}) on the basolateral membrane voltage (V_b) can then be calculated from the ratio of the basolateral membrane resistance (R_{bl}) to the sum of the apical (R_a) and basolateral membrane resistances:

$$\Delta V_{bl}/\Delta V_{te} = R_{bl}/(R_a + R_{bl}).$$

Using the membrane resistances of Frömter (1982), which were measured in the presence of luminal glucose and amino acid ($R_a = 135\ \Omega\cdot cm^2$ and $R_{bl} = 92\ \Omega\cdot cm^2$), this equation gives a ratio of 0.41. Thus, in the studies performed with 25 meq/liter HCO₃, replacement of peritubular gluconate with Cl will hyperpolarize V_{bl} by 3.8 mV, while in the experiment with 5 meq/liter HCO₃, the same maneuver will hyperpolarize the cell by 4.3 mV.

then lead to a cell pH change of similar magnitude but opposite in direction to that observed with peritubular Cl addition. However, we found that luminal Cl addition (in the absence of peritubular Cl) had no effect on cell pH in the presence of acid or alkaline solutions ($[HCO_3]$ = 5 or 25 meq/liter) (Alpern, 1987). Thus, it is unlikely that circular currents account for the observed effect of the peritubular Cl concentration on cell pH.

If Cl transport is not coupled to HCO_3 transport electrically, then it must be coupled chemically. To examine whether this coupling also involves Na, as previously found for *Necturus* basolateral membrane (Guggino et al., 1983), we examined the effect of peritubular Cl addition in the absence of luminal and peritubular Na. Rat proximal tubule cells contain an apical membrane Na/H antiporter and a basolateral membrane electrogenic $Na/(HCO_3)_{n>1}$ symporter, both of which should counteract the cell pH change induced by Cl/HCO_3 exchange. The inhibition of these mechanisms by luminal and peritubular Na removal should amplify the effect of altered Cl/HCO_3 exchange rate on cell pH. We have previously demonstrated (Alpern and Chambers, 1986) that inhibition of the basolateral membrane $Na/(HCO_3)_{n>1}$ symporter markedly enhances the response of cell pH to changes in the Na/H antiporter rate. In the present studies, removal of luminal and peritubular Na decreased the effect of peritubular Cl on cell pH.

The transporter described by Guggino et al. (1983) exchanged Na and HCO_3 for Cl. If the Na-dependent Cl/HCO_3 exchanger described in these studies is similar to that described by Guggino et al., the requirement for Na would be expected to be on the cell side of the basolateral membrane when Cl is added to the peritubular surface. In agreement with this prediction, our results that Na removal from the lumen or capillaries inhibited Cl/HCO_3 exchange are most consistent with a dependence on cell Na concentration. It is not possible from the data to state whether the magnitudes of inhibition seen with Na removal from the lumen and capillaries were greater than or equal to that seen with Na removal from the lumen or capillaries separately.

The dependence of Cl/HCO_3 exchange on Na may be due to effects other than Na coupling. One possibility is that Na removal leads to an elevation of the cell Ca concentration by Na/Ca exchange. The increased cell Ca concentration could then inhibit Cl/HCO_3 exchange. However, this thesis is not consistent with inhibition of Cl/HCO_3 exchange by luminal Na removal. Na/Ca exchange is currently believed to be present on the basolateral membrane of the proximal tubule, but not on the apical membrane. Lorenzen et al. (1984) found that peritubular Na removal in *Necturus* proximal tubule increased the cell Ca concentration, but luminal Na removal lowered the cell Ca concentration (Lorenzen et al., 1985). The possibility of another type of Na dependence, however, cannot be eliminated by the present studies.

The results are most consistent with the thesis that there are two modes of coupling between Cl and HCO_3. Since SITS inhibits Cl/HCO_3 exchange in the presence of Na, both modes are stilbene sensitive. One mode is inhibited by Na removal (Na dependent) and may represent an Na-coupled Cl/HCO_3 exchanger. The other is simple Cl/HCO_3 exchange, which continues in the absence of Na.

While we cannot rule out the possibility of incomplete Na removal in these studies, the finding of an Na-independent Cl/HCO₃ exchange in isolated basolateral membrane vesicles (Grassl et al., 1985) makes this explanation unlikely.

The relationship between the presently described Na-dependent Cl/HCO₃ exchanger and the previously described basolateral membrane Na-coupled electrogenic HCO₃ transport mechanism (Boron and Boulpaep, 1983; Alpern, 1985; Biagi, 1985; Yoshitomi et al., 1985; Grassl and Aronson, 1986; Akiba et al., 1986; Sasaki et al. 1986) was also addressed in these studies. In our previous studies (Alpern, 1985), we had not eliminated the possibility that the Na/(HCO₃)$_{n>1}$ transporter was coupled to Cl. We found in the present studies, however, that total removal of Cl from the luminal and peritubular fluid did not affect the response of cell pH to lowering the peritubular Na concentration. The present studies thus show that the major effect of the peritubular Na concentration on cell pH is through a Cl-independent transporter. This agrees with studies on rabbit basolateral membranes (Akiba et al., 1986) and on salamander proximal tubule (Boron and Boulpaep, 1983), where Na/HCO₃ cotransport was Cl independent.

Basolateral membrane Cl/HCO₃ exchange has been found in the turtle urinary bladder (Fisher et al., 1983) and in the cortical and outer medullary collecting ducts (Stone et al., 1983; Schwartz et al., 1985). Nakhoul and Boron (1985a) and Sasaki et al. (1986) have also reported preliminary studies on rabbit proximal straight tubules in which changes in the peritubular Cl concentration affect cell pH in a manner consistent with basolateral membrane Cl/HCO₃ exchange.

Guggino et al. (1983) demonstrated an Na-coupled Cl/HCO₃ exchanger on the basolateral membrane of the *Necturus* proximal tubule. These authors showed that Na or HCO₃ concentration gradients could drive the countertransport of Cl, and concluded that Na and HCO₃ were transported in one direction and Cl was transported in the opposite direction. Although it was not established in these studies whether this transporter was electrogenic or electroneutral, the many electrophysiologic studies described above (Shindo and Spring, 1981; Guggino et al., 1982; Cassola et al., 1983; Burckhardt et al., 1984) suggest that basolateral membrane Cl transport is electroneutral. If electroneutral, this transporter may be similar to the Na(HCO₃)₂/Cl exchanger described in invertebrate cells (R. C. Thomas, 1977; Boron et al., 1981) [this transporter has also been described as an Na(CO₃)/Cl or NaHCO₃/HCl exchanger].

Studies on basolateral membrane vesicles have identified anion exchangers that were able to function as Cl/HCO₃ exchangers. Grassl et al. (1985) found that these transporters did not require Na and were unaffected by Na addition. Low et al. (1984) found that the exchanger could function as an electroneutral, Na-independent anion exchanger or as an electroneutral Na/anion cotransporter. We have identified Na-independent and Na-dependent Cl/HCO₃ exchange. It is not presently clear whether this involves one transporter operating in two modes or two separate transporters. The Cl/HCO₃ exchanger in the red blood cell has been demonstrated to function also as an Na(HCO₃)₂/Cl exchanger (Becker and Duhm, 1978; Funder et al., 1978).

NaCl is absorbed by a transcellular electroneutral mechanism in the mamma-

lian PCT (Green et al., 1979; Baum and Berry, 1984; Alpern et al., 1985; Howlin et al., 1986). The present results provide a possible mechanism by which Cl absorbed across the apical membrane can be transported out of the cell across the basolateral membrane. On the basis of quantitative estimates of cellular ionic composition (Cassola et al., 1983; Yoshitomi and Frömter, 1985), a Cl/HCO_3 exchanger would not provide a mechanism for Cl efflux from the cell but rather would function in the opposite direction (HCO_3 efflux, Cl influx). An $Na(HCO_3)_2/Cl$ exchanger, however, would function in the Cl efflux direction.[2] The Na and HCO_3 that entered the cell in exchange for Cl could then leave the cell on the basolateral membrane $Na/(HCO_3)_{n>1}$ symporter. These studies do not address the role of the Na-dependent and -independent Cl/HCO_3 exchangers in basolateral membrane Cl transport. A neutral KCl symporter has been identified in isolated basolateral membrane vesicles (Eveloff et al., 1985), which may also contribute to cell Cl efflux. The transporters described here may also participate in the defense of cell pH (R. C. Thomas, 1977; Boron et al., 1981; Roos and Boron, 1981).

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[2] Given cell Na and Cl activities of 13 meq/liter (Cassola et al., 1983; Yoshitomi and Frömter, 1985), a cell pH of 7.2 ($[HCO_3]$ = 19 meq/liter at P_{CO_2} = 50 mmHg), extracellular Na and Cl activities of 102 and 83, and an extracellular HCO_3 concentration of 24 meq/liter, the free energy (ΔG) for Cl efflux across a Cl/HCO_3 exchanger is:

$$\Delta G = RT\ln[(83 \times 19)/(13 \times 24)] = 4,176 \text{ J/mol}.$$

Because ΔG is positive, this process will not proceed spontaneously (without energy input). A similar calculation for an $Na(HCO_3)_2/Cl$ exchanger is:

$$\Delta G = RT\ln[(83 \times 13 \times 19^2)/(13 \times 102 \times 24^2)] = -1,735 \text{ J/mol}.$$

This transporter will spontaneously transport Cl out of the cell.

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