Regulation of Net Bicarbonate Transport in Rabbit Cortical Collecting Tubule by Peritubular pH, Carbon Dioxide Tension, and Bicarbonate Concentration

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Abstract

The effects of changes in peritubular pH, carbon dioxide tension (PCO₂), and HCO₃⁻ concentration on net HCO₃⁻ transport was examined in vitro perfused cortical collecting tubules (CCTs) from unpretreated New Zealand white rabbits. Lowering peritubular HCO₃⁻ concentration and pH by reciprocal replacement of HCO₃⁻ with Cl⁻, significantly stimulated net HCO₃⁻ absorption. Lowering peritubular HCO₃⁻ concentration and pH, by substitution of HCO₃⁻ with gluconate, while keeping Cl⁻ concentration constant, also stimulated net HCO₃⁻ absorption. Raising peritubular HCO₃⁻ concentration and pH, by reciprocal replacement of Cl⁻ with HCO₃⁻, inhibited net HCO₃⁻ absorption (or stimulated net HCO₃⁻ secretion). When the tubule was cooled, raising peritubular HCO₃⁻ concentration had no effect on net HCO₃⁻ transport, suggesting these results are not due to the passive flux of HCO₃⁻ down its concentration gradient.

The effect of changes in ambient PCO₂ on HCO₃⁻ transport were also studied. Increasing the ambient PCO₂ from 40 mmHg to either 80 or 120 mmHg, allowing pH to fall, had no effect on net HCO₃⁻ transport. Similarly, lowering ambient PCO₂ to 14 mmHg had no effect on net HCO₃⁻ transport. Simultaneously increasing peritubular HCO₃⁻ concentration and PCO₂, without accompanying changes in peritubular pH, i.e., isohydric changes, stimulated net HCO₃⁻ secretion to the same degree as nonisohydric increases in peritubular HCO₃⁻ concentration. Likewise, isohydric lowering of peritubular HCO₃⁻ concentration and PCO₂ stimulated net HCO₃⁻ absorption.

We conclude that: (a) acute changes in peritubular HCO₃⁻ concentration regulate acidification in the CCT and these effects are mediated by a transcellular process; (b) acute changes in ambient PCO₂ within the physiologic range have no effect on HCO₃⁻ transport in the in vitro perfused CCT; and (c) acute in vitro regulation of CCT acidification is independent of peritubular pH.

Introduction

Whole-animal studies have suggested a role for the distal nephron in the renal response to systemic acid-base disturbances (1–3). Isolated perfused tubule studies have demonstrated that the cortical collecting tubule (CCT) can display either net HCO₃⁻ absorption or secretion (4–9). Thus, the CCT may participate in the final acidification or alkalization of the urine. The parameters modulating net HCO₃⁻ transport in the CCT have been only partially characterized. Isolated perfused tubule studies demonstrate that the direction of net HCO₃⁻ transport by the CCT is influenced by the preexisting acid-base status of the animal from which the tubule was harvested (4, 5, 7). Tubules harvested from animals with chronic metabolic acidosis demonstrate increased HCO₃⁻ absorption (4, 5, 7, 8), whereas tubules harvested from animals with chronic metabolic alkalosis display enhanced HCO₃⁻ secretion (4, 5, 9, 10). Thus, transport of HCO₃⁻ by the CCT can be influenced by chronic in vivo metabolic acid-base disturbances and furthermore appears to display a memory of the in vivo environment after being transferred to an in vitro system. The signal for this change in both the magnitude and direction of net HCO₃⁻ transport by the CCT is unknown.

Respiratory acid-base disorders may also influence CCT acidification. Recent morphologic studies focusing on the CCT intercalated cell have suggested a role for CO₂ in the regulation of CCT HCO₃⁻ absorption. Of the two cell types identifiable by light microscopy, it is the intercalated, or mitochondrial-rich cell, rather than the principal cell that is thought to reabsorb HCO₃⁻ via active H⁺ secretion (6, 11–17). Electron microscopy of this cell shows significant increases in the apical cell membrane surface area when experimental animals were subjected to 4 h of respiratory acidosis (16, 17). These changes have been interpreted as consistent with an increased number of proton pumps on the luminal membrane of the intercalated cell (16, 17). It has been suggested that this leads to augmented proton secretion by the CCT. Similar findings have been inferred in the turtle bladder and isolated perfused CCT (12, 18). Labeling of intracellular acid compartments with fluorescent probes, demonstrates augmented apical exocytosis in response to isohydric increases in ambient PCO₂. However, no measurements of HCO₃⁻ flux or proton secretion were made under conditions that examined isolated changes of PCO₂ and pH within the physiologic range.

Recent evidence suggests that the intercalated cell is also responsible for bicarbonate secretion (17, 19). It is felt that a subset of intercalated cells secrete HCO₃⁻ via apical cell membrane Cl⁻–HCO₃⁻ exchange (19, 20). As noted above, in vitro perfused CCTs can secrete and reabsorb HCO₃⁻. It is likely that both processes occur simultaneously and net HCO₃⁻ transport is the sum of these two unidirectional fluxes.

To date, net HCO₃⁻ transport by the isolated perfused rabbit CCT has been shown to be acutely influenced by transtubular Cl⁻ gradients (21), isoproterenol, and cyclic AMP (10). In addition, antidiuretic hormone has been shown to influence

1. Abbreviations used in this paper: CCT, cortical collecting tubule; PD, potential difference.

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HCO₃⁻ transport in the rat CCT (22). Direct acute effects of ambient acid-base conditions on net HCO₃⁻ transport in the CCT remain uncharacterized. This in vitro microperfusion study was therefore designed to examine the effect of acute, in vitro changes in peritubular HCO₃⁻ concentration, pH, and Pco₂, on net HCO₃⁻ transport by the CCT. These changes were designed to mimic the peritubular environment that might bathe the CCT during acute in vivo acid-base disorders. Our findings show that it is primarily peritubular HCO₃⁻ concentration but not peritubular pH or Pco₂ which influences net HCO₃⁻ transport in the CCT.

**Methods**

Female New Zealand white rabbits weighing between 1.5 and 2.0 kg were killed by decapitation. The left kidney was removed and 1-mm-thick coronal slices were made. These were placed in chilled ultrafiltrate-like solution containing 5% vol/vol fetal calf serum, pH 7.4. A slice was transferred in this chilled solution to a dissecting microscope where individual CCTs were freehand dissected with sharpened forceps. The freed segment was then transferred to a thermostatically controlled lucite bath chamber on an inverted microscopic stage. The tubule was cannulated and perfused with concentric micropipettes as previously described (4, 23, 24). The inner perfusion pipette was advanced 50–100 μm into the tubule lumen and served as a bridge into the tubular lumen to measure transepithelial potential difference (PD, in millivolts). Ringer’s agarose bridges were in contact with the perfusate in the rear of the pipette and with the bath solution. Each bridge was, in turn, connected to a calomel half-cell, via a second Ringer’s agarose bridge in series with the first. Transepithelial PD was monitored with an electrometer (Keithley Instruments, Inc., Cleveland, OH, model 602) and continuously recorded on a strip chart recorder.

After cannulation bath flow was adjusted to at least 0.5 ml/min with a Sage infusion pump (Sage Instruments, Cambridge, MA). The bathing solution was warmed to 37°C–38°C. The perfusion rate was adjusted to be between 0.5 and 2.0 nl/mm·min. The perfusate was collected in a constricted pipette of known volume that ranged between 14.5 and 36 nl. The equilibration period lasted 40–60 min after the bath heat was turned on. The control period was then begun with a measurement of volume flux (Ja), and then two to four total CO₂ flux (JtCO₂) determinations were made. After determination of control period Ja and JtCO₂, the bath was changed to one of 10 different experimental solutions. Another Ja determination was made, lasting 10–15 min. Then two to four additional determinations of JtCO₂ were made during the experimental period. These results were also averaged. The perfusate remained unchanged in all of these studies except for the assumed rapid equilibration of the bath and luminal Pco₂ (25) when a change in bath Pco₂ was made.

**Solutions**

In the majority of experiments the tubule was initially perfused and bathed in control solution. The control solution was an artificial ultrafiltrate-like solution with the following composition (in millimolar): NaCl 105, KCl 5, NaHCO₃ 25, Na acetate 10, NaHPO₄ 2.3, CaCl₂ 1.8, MgSO₄ 1, glucose 8.3, alanine 5. The control perfusate and bath were identical except that the bath also contained 5% vol/vol fetal calf serum and the perfusate contained exhaustively dialyzed tritiated inulin as a volume flux marker. Both of these solutions were equilibrated at 37°C in 95% O₂/5% CO₂ gas mixture to achieve a pH of 7.40.

The effects of ten different experimental conditions on Ja and JtCO₂ were examined. Their composition is shown in Table I. These solutions were designed to examine the effects of three different maneuvers on CCT HCO₃⁻ transport: (a) changing peritubular HCO₃⁻ concentration, (b) changing ambient Pco₂, (c) isohydric changes in HCO₃⁻ concentration and Pco₂.

**Group 1: effect of peritubular HCO₃⁻ concentration.** The tubule was equilibrated in the 25 mM HCO₃⁻ control bath. Control measurements were made, and then the bath was changed to either the 5, 10, or 50 mM HCO₃⁻-containing bath. Changes in peritubular HCO₃⁻ concentration were achieved by reciprocally changing peritubular Cl⁻ concentration except in the 10 mM HCO₃⁻ experiment where the peritubular HCO₃⁻ concentration was lowered by replacement with gluconate. This latter group of experiments was designed to measure the effect of peritubular HCO₃⁻ concentration in the absence of transtubular Cl⁻ concentration gradients.

The contribution of passive movement of HCO₃⁻ down a concentration gradient across the CCT was also examined. In this study the control solutions were symmetrical 25 mM HCO₃⁻-containing solutions, which were identical to the previous control solutions except that 25 mM of Cl⁻ were replaced with gluconate so the control bath and peritubular Cl⁻ concentration were 90 mM. This allowed us to impose a 25 mM bath to lumen HCO₃⁻ gradient without imposing any Cl⁻ gradient. In this protocol we first measured JtCO₂ at 37°C. The bath was then cooled to 20°C using a water-jacketed bath line. JtCO₂ was again measured. Finally, the bath was changed to a 50 mM HCO₃⁻, Pco₂ 40, pH 7.70 solution identical to the metabolic alkalosis bath but at 20°C. JtCO₂ was again measured. In all of these experiments the liquid junction potential was measured as <0.5 mV. This correction did not significantly change the results of the transepithelial PD and so was discounted.

**Group 2: effects of changing ambient Pco₂.** In addition to the control Pco₂ of 40 mmHg, we varied ambient Pco₂ from values as low as 8 mmHg up to 120 mmHg. Ambient pH was allowed to vary.

**Group 3: isohydric changes in ambient Pco₂ and HCO₃⁻ concentration.** These studies examined the effect of changing the Pco₂ and HCO₃⁻ concentration.

![Table I. Composition of Solutions](https://example.com/table1.png)

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<th>Solutions</th>
<th>pH</th>
<th>Pco₂</th>
<th>HCO₃⁻</th>
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</tr>
<tr>
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<td>115</td>
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<td>8</td>
<td>25</td>
<td>115</td>
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<td>7.4</td>
<td>10</td>
<td>130</td>
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</table>

The composition of the constituents of the bathing solutions (in millimolar) which were changed in each experimental group are listed. All solutions also contained (in millimolar): Na⁺ 145, K⁺ 5, Mg²⁺ 1, acetate 10, glucose 8, alanine 5, HPO₄⁻ 2.3, and Ca²⁺ 1.8. The bath also contained 5% vol/vol fetal calf serum while the perfusate contained exhaustively dialyzed inulin.

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centration simultaneously so that bath pH did not vary. In this group bath HCO₃⁻ concentration was changed by reciprocally altering Cl⁻ concentration. In the control period the bath, as usual, contained 25 mM HCO₃⁻, Pco₂ 40 mmHg, pH 7.40. The experimental solutions examined either an isohydric increase in HCO₃⁻ concentration (50 mM, Pco₂ 80, pH 7.40) or an isohydric decrease in HCO₃⁻ concentration (10 mM, Pco₂ 18 mmHg, pH 7.40).

Microassays

Net volume flux (Jv) was determined from changes in the [³H]inulin activity between the collected fluid and the perfusate as previously described (24, 26, 27). This was used predominantly to rule out any bulk leak of fluid across the tubule or any significant water flux. Any tubule with a Jv greater than ±0.05 nl/mm·min was discarded.

Jtco₂ was determined by microcalorimetry (28) and calculated according to the equation: Jtco₂ = ([CO₂]in – [CO₂]out) · V̇w/min·l/length (mm). V̇w/min equals the rate the perfusate was collected. This equation assumes negligible change in HCO₃⁻ concentration due to water flux (as excluded by the Jv determinations). Samples were injected immediately after collection into concentrated sulfuric acid in the picnograph chamber (University of California Research Dept.). This value was then compared to a paired injection of the 25 mM Na₂CO₃ standard. The Jtco₂ measurements for each period were averaged and this value represents one data point in each individual experiment. At the end of each experiment the tubule was released and the perfusion pipette was advanced directly into a collection pipette. The perfuse total CO₂ (tco₂) and [³H]inulin content were then assayed using the same constant volume perfusate as used during the experiment. The perfusate tco₂ was measured two to four times and the values averaged. The measured tco₂ was the value used for [CO₂]out. The calibration performed at the end of each experiment was assayed for internal variation. The mean standard deviation was 0.8 mM. The average number of measurements made was 3.26 per each experimental period. Thus, the accuracy of each average tco₂ collection per period is 0.8 mM/√3.26 (i.e., standard error of the mean) or 0.44 mM (29). Thus, we could reliably determine differences of 0.88 mM tco₂ between experimental periods.

In those experiments where ambient Pco₂ was altered, the tco₂ of the collected perfusate was corrected for the contribution that the change in Pco₂ would theoretically make in measured total CO₂. Thus if the Pco₂ of the bath was raised from 40 to 80 mmHg between control and experimental periods the tco₂ of the collected perfusate would be expected to increase 0.03 · Pco₂ (mmHg) or, in this case, 1.2 mM. Therefore, 1.2 mM was subtracted from the measured tco₂ in each of the experimental period collections. Because there is some loss of CO₂ into the oil in the collection pipette this correction tends to slightly overestimate increases in HCO₃⁻ absorption and underestimate increases in HCO₃⁻ secretion.³

In studies with two periods the Jtco₂ for each tubule was compared between periods by a two-tailed, paired Student’s t test. For those experiments where three periods were examined, comparison was made by analysis of variance. P values <0.05 were considered significant.

Results

Effect of changing peritubular HCO₃⁻ concentration. The peritubular environment during acute metabolic acidosis was simulated by lowering peritubular pH from 7.4 to 6.96 and the bath HCO₃⁻ concentration from 25 to 5 mM. Bath HCO₃⁻ was replaced milliequivalent for milliequivalent with Cl⁻. The control perfusion rate was 1.72±0.10 nl/mm·min and 1.53±0.07 nl/mm·min in the experimental period (P = 0.10, NS). In five tubules averaging 1.74±0.14 mm in length, the average trans-epithelial PD was −22.6±3.8 mV during the control period and −21.6±3.9 mV during the experimental period (P = 0.60, NS). The mean Jtco₂ was 2.8±1.3 pmol/mm·min during the control vs. 5.1±1.2 pmol/mm·min during the experimental period (P < 0.02) (Fig. 1, Table 1). Thus lowering the peritubular HCO₃⁻ and pH significantly stimulates HCO₃⁻ absorption in the CCT.

We also attempted to simulate the peritubular environment during acute metabolic alkalosis by raising the peritubular HCO₃⁻ concentration from 25 to 50 mM, raising pH from 7.4 to 7.7. Control and experimental period perfusion rates were

<table>
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<th>Control</th>
<th>Metabolic Acidosis</th>
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<tr>
<td>pH</td>
<td>HCO₃⁻</td>
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<tr>
<td>7.4</td>
<td>25</td>
</tr>
<tr>
<td>7.4</td>
<td>25</td>
</tr>
</tbody>
</table>

Figure 1. In vitro peritubular metabolic acidosis increases net HCO₃⁻ absorption in the CCT. Individual tubules (n = 5) (●). Mean Jtco₂±SE in pmol/mm·min·c (●). Control 2.8±1.3 vs. experimental 5.1±1.2, P < 0.02.

3. Preliminary experiments documented loss of CO₂ into the oil phase with time; previous work (26) has shown that the correction for Pco₂ between 14 and 40 mmHg is correct; that the correction for Pco₂ between 40 and 80 mmHg is off by only 0.4 mM; and that the use of CO₂-equilibrated oil retards the loss of CO₂ from the collected perfusate. In the group of experiments examining the effect of 120 mmHg Pco₂ we used mineral oil equilibrated with 5% and 15% CO₂ behind the collected perfusate during control and experimental periods, respectively. The increase in tco₂ owing to the increase in dissolved CO₂ was also directly measured. This measurement was performed at the end of each experiment by inhibiting active transport. We achieved this by bathing the tubule in 10⁻⁴ M acetazolamide and rapidly perfusing the tubule. Acetazolamide inhibits active transport of HCO₃⁻ in this segment (8, 9) and rapid perfusion makes any potential residual active transport of HCO₃⁻ in this segment, undetectable (4). Cooling could not be used to inhibit transport in this case because it would change the solubility of CO₂ in oil and aqueous phase. We documented the inhibition of active transport by this technique by comparing the tco₂ collected from a tubule treated in this manner (in a Pco₂ of 40 mmHg) and the tco₂ collected directly from the perfusion pipette. No difference was found.

After rapidly filling the collection pipette with a volume of perfusate roughly equal to the sample pipette volume, the perfusate was allowed to sit in the collection pipette at 37° C for 10 min (the average collection time). The collected tco₂ was then measured. The difference in tco₂ measured when the acetazolamide-treated tubule was perfused with an ambient Pco₂ of 120 vs. 40 mmHg, averaged 2.0 mM. This number was used to correct for the contribution that difference in dissolved CO₂ makes to the change in tco₂ collected during each period. Correction of the measured collected tco₂ for the theoretical contribution that titration of HCO₃⁻ by luminal phosphate makes to tco₂, does not change the statistical significance of our data.
1.92±0.34 and 1.98±0.23 pmol/mm·min, respectively, (P = 0.59, NS). The average PD during the control period was −17.6±5.9 mV and −18.4±6.0 (P > 0.60, NS) during the experimental period. Fig. 2 and Table II show the results for seven tubules with a length of 2.15±0.17 mm. Net HCO3− secretion was stimulated or absorption inhibited by raising peritubular HCO3− concentration (control −0.7±2.4 vs. experimental −4.5±1.4 pmol/mm·min, P < 0.05). Thus metabolic alkalosis stimulates net HCO3− secretion in the CCT.

Because in both of these experiments transtubular Cl− as well as HCO3− concentration gradients existed, we examined the effect of lowering the bath HCO3− concentration without simultaneously imposing a Cl− gradient (Fig. 3, Table II). Peritubular HCO3− concentration was lowered to 10 mM by replacement with gluconate. This study simulates the peritubular environment during an acute anion gap acidosis. The pH was 7.4 during the control period and 7.05 during the experimental period. Control and experimental period perfusion rates were 0.85±0.08 and 0.84±0.08 nl/mm·min, respectively (P = 0.84, NS). The mean PD during the control period was −14.1±4.5 vs. −17±6.8 mV during the experimental period (P > 0.40, NS). Fig. 3 shows the results for 10 tubules averaging 2.13±0.12 mm in length. Net HCO3− absorption increased in the period of “anion gap acidosis” as compared with the control period (control 2.21±0.74 pmol/mm·min vs. experimental 4.38±0.66 pmol/mm·min, P < 0.0005).

This group also contains a time control because in six tubules the sequence of bath change was from control to experimental and in four tubules this sequence was reversed. In the six tubules changing from control to experimental mean JTCO2 increased from 2.12±1.22 to 4.38±0.86 pmol/mm·min (P < 0.005). The change in JTCO2 in the four experimental-to-control tubules was 4.40±1.07 to 2.35±0.61 pmol/mm·min (P < 0.05). These results show that changes in peritubular HCO3− and pH can modulate HCO3− transport in the isolated perfused CCT. Furthermore these effects are not dependent on accompanying changes in peritubular Cl− concentration.

To determine if the passive flux of HCO3− down its concentration gradient could contribute to our results, we cooled the tubule to 20°C and then measured net HCO3− transport before and after imposing a 25 mM HCO3− gradient from bath to lumen (Fig. 4, Table II). No transepithelial Cl− gradient existed in these experiments. In the first period net HCO3− transport was measured at 37°C. The perfusion rate was unchanged between periods (control 0.59±0.09, experimental 0.56±0.06, recovery 0.59±0.03 nl/mm·min). In four tubules with a mean length of 2.41±0.06 mm, control PD averaged −12.0±6.4 mV and JTCO2 was 1.32±0.40 pmol/mm·min. The tubules were then cooled to 20°C. PD fell to −5.3±3.4 mV (control vs. experimental, P > 0.10, NS) and JTCO2 fell to −0.10±0.31 pmol/mm·min, a value not statistically different from 0. The peritubular HCO3− concentration was then increased from 25 to 50 mM by reciprocal replacement of bath gluconate. The mean PD depolarized slightly more to 1.25±1.3 mV (P > 0.10 compared with both control and experimental periods, NS)). The mean JTCO2 was unchanged (0.20±0.14 pmol/mm·min, P > 0.30 compared with previous period). Thus, there is no change in JTCO2 when a 25 mM bath to lumen HCO3− gradient is imposed on CCTs in which active transport is inhibited by cooling to 20°C.

Effect of changing ambient Pco2. In experiments designed to simulate acute respiratory acid-base disorders, the bath Pco2 and pH were altered. Two different degrees of acute in vitro respiratory acidosis and respiratory alkalosis were studied.

Ambient pH was lowered from 7.4 to 7.05 by raising the ambient Pco2 from 40 to 80 mmHg (Fig. 5, Table II). Control and experimental period perfusion rates were 1.06±0.08 and 1.09±0.04 nl/mm·min, respectively (P = 0.61, NS). In six tubules with a mean length of 2.06±0.14 mm, the PD averaged −31.7±4.3 mV during the control period and −29.8±3.9 mV during the period of hypercapnea (P > 0.60, NS). The mean JTCO2 during the control period was 1.44±0.74 pmol/mm·min and 2.36±1.50 pmol/mm·min during the experimental period. This difference was not statistically significant (P > 0.40). Thus, doubling the Pco2 and lowering the pH had no effect on net HCO3− transport.

Micropuncture studies in the rat have shown that during acute in vivo respiratory acidosis renal cortical Pco2 may be as much as 50 mmHg greater than the arterial Pco2 (30). Renal cortical Pco2 in the rabbit has also been found to be roughly 20 mmHg higher than arterial Pco2 under eucapnic conditions (31). Furthermore, a variety of studies suggest that acute in vivo respiratory acidosis stimulates HCO3− absorption in the distal neprhon and CCT in particular (3, 17).

It is possible that doubling the bath Pco2 might be insufficient to evoke a measurable increase in HCO3− absorption. We therefore examined the effect of raising bath Pco2 from 40 to 120 mmHg, on net TCO2 flux in the CCT. The ambient pH was changed from 7.40 in the control period to 6.92 during the experimental period. In three of five tubules the sequence of bath change was from control to experimental and in two of five tubules the sequence of bath change was from experimental to control. Control and experimental period perfusion rates were 0.90±0.17 and 0.87±0.17 nl/mm·min, respectively (P = 0.82, NS). The acid, high Pco2 bath consistently depolarized the transepithelial PD. In these five tubules, averaging 2.08±0.17 mm in length, the mean PD in the control bath was −11.8±6.8 mV vs. 0.0±1.5 mV in the high Pco2 bath.
Table II. Microperfusion Data

<table>
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<th>Perfusion rate</th>
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<th>Collected TCO₂</th>
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<td>23.0±0.71</td>
<td>1.32±0.4</td>
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<tr>
<td></td>
<td>E 5</td>
<td>0.56±0.06</td>
<td>25.1±0.45</td>
<td>-0.10±0.3</td>
<td>0.20±0.1</td>
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<tr>
<td></td>
<td>R 12</td>
<td>0.59±0.03</td>
<td>25.2±0.33</td>
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<tr>
<td>Moderate respiratory acidosis</td>
<td>C 6</td>
<td>1.06±0.08</td>
<td>24.4±0.20</td>
<td>22.8±0.74</td>
<td>1.44±0.7</td>
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<tr>
<td></td>
<td>E 7</td>
<td>1.06±0.04</td>
<td>22.3±1.15</td>
<td>2.36±1.5</td>
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<td>Severe respiratory acidosis</td>
<td>C 5</td>
<td>0.90±0.17</td>
<td>25.5±0.34</td>
<td>25.7±0.95</td>
<td>-0.96±1.12</td>
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<tr>
<td></td>
<td>E 6</td>
<td>0.87±0.17</td>
<td>25.5±1.88</td>
<td>-0.86±1.38</td>
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<td>Moderate respiratory alkalosis</td>
<td>C 7</td>
<td>1.07±0.10</td>
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<td>E 8</td>
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<td>20.9±1.00</td>
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<td>Severe respiratory alkalosis</td>
<td>C 3</td>
<td>0.80±0.11</td>
<td>23.8±0.33</td>
<td>18.1±1.99</td>
<td>3.91±0.71</td>
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<tr>
<td></td>
<td>E 4</td>
<td>0.80±0.10</td>
<td>23.2±0.53</td>
<td>0.30±0.21</td>
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<tr>
<td></td>
<td>R 13</td>
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<td>20.9±0.97</td>
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<tr>
<td>Isohydric increase HCO₃</td>
<td>C 5</td>
<td>0.93±0.08</td>
<td>24.3±0.16</td>
<td>27.4±0.89</td>
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<td>E 6</td>
<td>1.18±0.09</td>
<td>31.0±1.72</td>
<td>-6.83±1.31</td>
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<td>R 14</td>
<td>0.92±0.05</td>
<td>27.2±0.97</td>
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<td>Isohydric decrease HCO₃</td>
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<td>R 15</td>
<td>0.67±0.08</td>
<td>22.0±1.16</td>
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C, control period; E, experimental period; R, recovery period. Values given as mean±SE. JTCO₂ is net TCO₂ transport. * P < 0.05. § P < 0.01. § P < 0.001.

(P > 0.10, NS). Again, as in the study on the effect of raising the ambient Pco₂ to 80 mmHg, no significant change in JTCO₂ was observed between the periods of eucapnea and hypocapnea to 120 mmHg (Fig. 6, Table II). The mean JTCO₂ was -0.96±1.12 pmol/mm · min during control and -0.86±1.38 pmol/mm · min during the experimental period. Analysis of the pooled data for both groups (n = 11) studying the effect of hypocapnea on JTCO₂ also failed to reveal a significant effect of this maneuver (P > 0.40). Thus, acidosis due to hypocapnea does not stimulate HCO₃⁻ absorption in the isolated perfused CCT.

Moderate in vitro respiratory alkalosis was also studied (Fig. 7, Table II). The ambient Pco₂ was lowered from 40 to 14 mmHg and bath pH increased from 7.4 to 7.8. Control and experimental period perfusion rates were 1.07±0.10 and 1.10±0.12 nl/mm · min, respectively (P < 0.82, NS). In seven tubules, with a mean length of 1.91±0.10 mm, the PD was unchanged (-20.9±4.4 mV during control and -19.7±3.02 mV during experimental period, P > 0.60, NS). There was no difference between control JTCO₂ and that observed during hypocapnea being 3.11±1.23 vs. 3.27±1.34 pmol/mm · min respectively. Thus, moderate degrees of hypocapnea do not affect HCO₃⁻ transport in the CCT.

Because CO₂ has been shown to be important to HCO₃⁻ transport in a variety of acidifying epithelia including the CCT and turtle bladder (11, 12, 15, 24, 25, 32-34), we examined a more severe degree of in vitro respiratory alkalosis to look for an inhibitory effect of hypocapnea on CCT HCO₃⁻ transport. The bath Pco₂ was acutely lowered to 8 mmHg (Fig. 8, Table II).

During acute in vivo respiratory alkalosis in the rat, renal cortical Pco₂ was directly measured as 25 mmHg at a time when arterial Pco₂ was 15 mmHg (30). Even if an animal could be hyperventilated to an arterial Pco₂ of 8 mmHg, the renal cortical Pco₂ would not be this low. This degree of hypocapnea is thus well outside the physiologic range.

Raising the ambient pH and lowering the Pco₂ to 8 mmHg depolarized the transepithelial PD from -30±0.0 mV to -18.5±1.5 mV (NS). The perfusion rate was unchanged (control 0.80±0.11, experimental 0.80±0.10, recovery 0.7±0.05 nl/mm · min, P > 0.48 in all comparisons). In three tubules, with
a mean length of 2.15 ± 0.05 mm, lowering the PCO₂ to 8 mmHg significantly suppressed HCO₃⁻ transport from 3.91±0.71 to 0.30±0.21 pmol/mm · min (P < 0.025). Return to control bath was associated with partial recovery of HCO₃⁻ transport to 2.01±0.55 pmol/mm · min. This recovery value was statistically different from the control HCO₃⁻ flux (P < 0.05). Thus, although respiratory acidosis and moderate respiratory alkalosis do not measurably affect HCO₃⁻ transport in the CCT, severe reduction of the PCO₂ does result in inhibition of HCO₃⁻ transport.

**Effects of isohydric changes in PCO₂ and HCO₃⁻ concentration.** By simultaneously changing bath HCO₃⁻ concentration and PCO₂ we were able to vary peritubular HCO₃⁻ concentration without

![Figure 3](image1.png)

*Figure 3.* In vitro peritubular anion gap metabolic acidosis stimulates net HCO₃⁻ absorption in the CCT. Peritubular [HCO₃⁻] was lowered by replacing HCO₃⁻ with gluconate. Individual tubules (n = 10) (o). The arrows indicate the sequence of bath change. Mean JₜCO₂±SE in pmol/mm · min (o). Control 2.21±0.74 vs. experimental 4.38±0.66, P < 0.0005.

![Figure 4](image2.png)

*Figure 4.* Raising bath [HCO₃⁻] does not affect net HCO₃⁻ transport in tubes cooled to 20°C. Bath [HCO₃⁻] was increased without changing [Cl⁻] by substituting it for the 25 mM gluconate present in control bath. Individual tubules (n = 4) (o). Mean JₜCO₂±SE in pmol/mm · min. Period 1, 1.32±0.40; period 2, 0.10±0.31; period 3, 0.20±0.14. Period 1 vs. 2 P < 0.10. Period 2 vs. 3 P < 0.30. Period 2 vs. 0 P < 0.70.

![Figure 5](image3.png)

*Figure 5.* In vitro moderate respiratory acidosis (PCO₂ = 80 mmHg) has no effect on net HCO₃⁻ transport in the CCT. Individual tubules (n = 6) (o). Mean JₜCO₂±SE in pmol/mm · min (o). Control 1.44±0.74 vs. experimental 2.36±1.50, P > 0.40.

![Figure 6](image4.png)

*Figure 6.* In vitro severe respiratory acidosis (PCO₂ = 120 mmHg) has no effect on net HCO₃⁻ transport in the CCT. Individual tubules (n = 5) (o). Mean JₜCO₂±SE in pmol/mm · min (o). Control −0.96±1.12 vs. experimental −0.86±1.38, P < 0.80.
varying pH. This maneuver enables one to determine whether the modulation of net $\text{HCO}_3^-$ transport by changes in peritubular $\text{HCO}_3^-$ and/or $\text{Cl}^-$ concentration was due to the effects of changing the peritubular anion concentrations or due to the accompanying change in pH.

We doubled the peritubular $\text{HCO}_3^-$ concentration replacing $\text{Cl}^-$ milliequivalent for milliequivalent. The peritubular pH was maintained constant at 7.40 by simultaneously doubling the ambient $\text{PCO}_2$ from 40 to 80 mmHg. The perfusion rate was unchanged except between experimental and recovery where there was a statistically significant difference, $P = 0.03$ (control $0.93\pm0.08$, experimental 1.18$\pm0.09$, recovery $0.92\pm0.05 \text{ nl/mm min}$). In five tubules averaging 2.17$\pm0.14$ mm, the PD in the control period averaged $-21.7\pm4.8 \text{ mV}$ vs. $-20.8\pm3.9 \text{ mV}$ ($P > 0.70$, NS) during the experimental period. Doubling the peritubular $\text{HCO}_3^-$ concentration without changing pH stimulated $\text{HCO}_3^-$ secretion in every case (Fig. 9, Table II). The mean $J_{\text{HCO}_3^-}$ in the control period was $-2.69\pm0.83 \text{ pmol/mm min}$ and increased to $-6.83\pm1.31 \text{ pmol/mm min}$ during the experimental period. This stimulation was fully reversible by returning to the control bath. The change in net $\text{HCO}_3^-$ flux observed with the isohydric increase in peritubular $\text{HCO}_3^-$ concentration was comparable to that observed with nonisohydric changes in bath $\text{HCO}_3^-$ concentration ($\Delta = -4.14 \text{ vs. } -3.80 \text{ pmol/mm min}$). Thus, raising bath $\text{HCO}_3^-$ stimulates net $\text{HCO}_3^-$ secretion whether the peritubular pH increases or remains constant.

The peritubular $\text{HCO}_3^-$ concentration was also isohydrically acutely lowered by replacing $\text{HCO}_3^-$ with $\text{Cl}^-$ milliequivalent for milliequivalent. The ambient $\text{PCO}_2$ was lowered to a value within a range of $\text{PCO}_2$ tensions shown, by earlier experiments, not to independently alter net $\text{HCO}_3^-$ transport in the CCT (i.e., $\geq 14 \text{ mmHg}$). The perfusion rate was unchanged (control $0.66\pm0.13$, experimental $0.65\pm0.14$, recovery $0.67\pm0.08 \text{ nl/mm min}$, $P \geq 0.84$ in all comparisons). In four tubules, averaging $2.08\pm0.17 \text{ mm}$ in length, the PD in the control period was $-13.3\pm3.9 \text{ mV}$ vs. a mean PD of $-15.0\pm6.9 \text{ mV}$ during the experimental period ($P > 0.60$, NS). Isohydrically lowering bath $\text{PCO}_2$ concentration to 10 mM and the $\text{PCO}_2$ to 18 mmHg significantly stimulated net $\text{HCO}_3^-$ absorption in four of four tubules from a mean $J_{\text{HCO}_3^-}$ of $0.21\pm0.57$ to $3.39\pm0.76 \text{ pmol/mm min}$ during the experimental period ($P < 0.005$) (Fig. 10, Table II). Changing back to the control bath was associated with a decrease in net $\text{HCO}_3^-$ absorption to $2.59\pm0.72 \text{ pmol/mm min}$ ($P < 0.08$). This
recovery was only partial since \( J_{\text{ICO}} \) was not significantly different from experimental \( J_{\text{ICO}} \) using analysis of variance. The reason for incomplete recovery is unclear but may be secondary to the small number of tubules in this group. Additionally, recovery may require a longer period of time. Nevertheless, this experiment is consistent with the previous studies and also demonstrates that lowering the bath HCO\(_3\) concentration stimulates net HCO\(_3\) absorption whether pH is constant or allowed to fall.

**Discussion**

The present studies examine the effect of acute in vitro changes in peritubular HCO\(_3\), pH, and Pco\(_2\) on net HCO\(_3\) transport in the CCT. Lowering the peritubular HCO\(_3\) and pH stimulates net HCO\(_3\) absorption whereas raising the peritubular HCO\(_3\) and pH stimulates net HCO\(_3\) secretion. Lowering peritubular HCO\(_3\) without changing peritubular Cl\(^-\) concentration also stimulated HCO\(_3\) absorption, showing that alterations in peritubular Cl\(^-\) concentration were not necessary for this effect. Cooling experiments were performed to show that the passive movement of HCO\(_3\) down its concentration gradient does not account for these findings. Changes in ambient Pco\(_2\), associated with changes in bath pH comparable to those studied in the metabolic acid-base disturbance protocols, had no effect on net HCO\(_3\) transport by the CCT. Only when the ambient Pco\(_2\) was lowered to \( \sim 8 \) mmHg was net HCO\(_3\) absorption inhibited. Finally, isohydric increases and decreases in HCO\(_3\) concentration demonstrate that changes in peritubular HCO\(_3\) but not pH was the critical parameter modulating net HCO\(_3\) transport. In conclusion we find that changes in peritubular HCO\(_3\) and Cl\(^-\) concentration, but not pH or ambient Pco\(_2\) (within the physiologic range), regulate net HCO\(_3\) transport in the CCT.

Bicarbonate movement in the CCT is unique in that bidirectional active HCO\(_3\) transport exists. McKinney and Burg (5) first demonstrated HCO\(_3\) transport in the CCT and documented that chronic in vivo metabolic alkalosis stimulates net HCO\(_3\) secretion whereas chronic in vivo metabolic acidosis stimulates net HCO\(_3\) absorption. Both of these processes are very similar to HCO\(_3\) transport mechanisms in the turtle bladder (8–11, 34–36).

Bicarbonate absorption by both the turtle bladder and the CCT is electrogenic (6, 11, 32–35). Most data suggest that protons are electrogenically pumped into the lumen by an apical membrane proton translocating adenosine triphosphatase leaving OH\(^-\) ions in the cell (35–39). Since HCO\(_3\) absorption is inhibited by low CO\(_2\) tensions and acetazolamide (8, 32–34, 36) it is likely that the intracellular OH\(^-\) is carboxylated by CO\(_2\), in a carbonic anhydrase facilitated step, to form HCO\(_3\), which then exits the cell. HCO\(_3\) absorption is inhibited by the removal of peritubular Cl\(^-\) so it has been postulated that basolateral HCO\(_3\) exit is mediated by a Cl\(^-\)/HCO\(_3\) exchanger (40, 41). Electrochemical gradients for both HCO\(_3\) and Cl\(^-\) may thus influence base exit from the cell.

The mechanism for unidirectional HCO\(_3\) secretion is less well established. This process appears to be sodium-independent, insensitive to ouabain, and inhibited by acetazolamide (9, 10, 20). HCO\(_3\) secretion is also inhibited by the removal of luminal Cl\(^-\), so that a luminal Cl\(^-\)/HCO\(_3\) exchanger has been proposed (10, 19, 20, 42, 43). It has recently been postulated that a basolateral proton translocating adenosine triphosphatase secretes protons into the peritubular space, raising cell HCO\(_3\) concentration, thus driving HCO\(_3\) secretion (19, 44). The HCO\(_3\) secretory cell may then be essentially the same as the HCO\(_3\) absorptive cell except that its polarity is reversed. According to this model the HCO\(_3\) secretory cell would possess a luminal Cl\(^-\)/HCO\(_3\) exchanger and basolateral proton pump. This is the inverse of the apical proton pump and basolateral Cl\(^-\)/HCO\(_3\) exchanger that has been proposed for the HCO\(_3\) absorptive cell. Net HCO\(_3\) transport in the CCT is the sum of these two unidirectional processes.

Our studies have demonstrated that changes in peritubular HCO\(_3\) and Cl\(^-\) concentration influence HCO\(_3\) transport by the CCT. Transtubular Cl\(^-\) gradients have been shown to influence net HCO\(_3\) secretion (21). Chloride gradients might alter net HCO\(_3\) transport in the CCT by influencing base exit via a Cl\(^-\)/HCO\(_3\) exchanger which could be located on either the luminal or peritubular membrane. Changes in Cl\(^-\) concentration gradients across membranes containing a proton pump may also influence the rate of proton pumping. For example, altered HCO\(_3\) absorption in the medullary collecting duct has been observed with changes in luminal Cl\(^-\) concentration (41). Removing luminal Cl\(^-\) in the medullary collecting duct stimulated HCO\(_3\) absorption by 50%. Because this segment only absorbs HCO\(_3\) (4) it is unlikely that inhibition of simultaneous HCO\(_3\) secretion accounts for these observations. It was postulated that the increased bath-to-lumen Cl\(^-\) gradient enhanced shunting of electrogenic proton secretion. Thus, if the HCO\(_3\) secretory cell is in fact the same as the HCO\(_3\) absorptive cell, except with reversed polarity, changes in peritubular Cl\(^-\) concentration may influence both unidirectional HCO\(_3\) secretion and unidirectional HCO\(_3\) absorption, thereby altering net HCO\(_3\) transport. Whatever the mechanism, it is unquestionable that Cl\(^-\) concentration gradients can influence net HCO\(_3\) transport in the CCT.

The changes in peritubular Cl\(^-\) concentration used in the current study are similar to the changes in peritubular Cl\(^-\) concentration that would be predicted to occur in vivo during met-
abolic acid-base disturbances. These gradients are smaller than those studied by Laski et al. (21). As is the case in many metabolic acid-base disorders, our studies reciprocally changed peritubular Cl\textsuperscript- concentration and HCO\textsubscript3\textsuperscript- concentration. Isolated changes in peritubular HCO\textsubscript3\textsuperscript- concentration would be expected to change transcellular HCO\textsubscript3\textsuperscript- flux if the above model is correct. Changing the HCO\textsubscript3\textsuperscript- concentration would alter the driving force across the Cl\textsuperscript-/HCO\textsubscript3\textsuperscript- exchanger in the proton secretory cell and might alter basolateral proton pumping in the HCO\textsubscript3\textsuperscript- secretory cell by changing the peritubular pH and the electrochemical driving force for H\textsuperscript+ pumping. Because we demonstrate in these studies that lowering the peritubular HCO\textsubscript3\textsuperscript- concentration without changing peritubular Cl\textsuperscript- concentration stimulates net HCO\textsubscript3\textsuperscript- absorption, our data verifies that Cl\textsuperscript- concentration is not the sole factor modulating HCO\textsubscript3\textsuperscript- transport in metabolic acid-base disturbances. These findings cannot distinguish this effect as being on unidirectional bicarbonate secretion, unidirectional bicarbonate absorption or both.

We examined the role of passive HCO\textsubscript3\textsuperscript- flux down its concentration gradient. Active HCO\textsubscript3\textsuperscript- transport was inhibited by cooling the tubule to 20\textdegreeC. A HCO\textsubscript3\textsuperscript- concentration gradient was then imposed. The results show no change in J\textsubscript\text{TCO}_2 when cooled tubules are exposed to changes in peritubular HCO\textsubscript3\textsuperscript- concentration. Under these conditions, in CCTs from untreated rabbits, estimated bicarbonate permeability is 0. Using intracellular microelectrodes, Samsom et al. (45) directly measured anion conductance in the isolated perfused rabbit CCT at 38\textdegreeC. They could not detect any HCO\textsubscript3\textsuperscript- conductance. Paracellular ionic movement is conductive in nature so the absence of HCO\textsubscript3\textsuperscript- conductance in this segment argues strongly against paracellular bicarbonate movement. In accord with these findings are the experiments in which P\textsubscript\text{CO}_2 was raised to 120 mmHg. In these studies a mean voltage drop from -11.8 to +1.5 mV had no effect on J\textsubscript\text{TCO}_2. This is in agreement with the lack of conductive movement of HCO\textsubscript3\textsuperscript- in this segment. The lack of an effect of HCO\textsubscript3\textsuperscript- concentration gradients in cooled CCTs also argues against passive paracellular movement of HCO\textsubscript3\textsuperscript- in the CCT. Hence changes in peritubular HCO\textsubscript3\textsuperscript- concentration modulate a transcellular transport process.

The next series of experiments examined the effects of altering ambient P\textsubscript\text{CO}_2 within the physiologic range. We found no effect of changing P\textsubscript\text{CO}_2, over a range from 14 to 120 mmHg, on net HCO\textsubscript3\textsuperscript- transport in the CCT. There are several possible explanations for this lack of an effect of changes in P\textsubscript\text{CO}_2 and pH on net bicarbonate transport in the CCT. It is possible that unidirectional bicarbonate secretion and unidirectional bicarbonate absorption may both be simultaneously, symmetrically, stimulated or inhibited by these changes in P\textsubscript\text{CO}_2. Both net bicarbonate secretion and bicarbonate absorption are inhibited by acetazolamide (8, 9). This suggests they may both be dependent on the presence of CO\textsubscript2 and carbonic anhydrase for the formation of HCO\textsubscript3 from intracellular OH\textsuperscript- ion and CO\textsubscript2 as outlined previously. The experimental changes in P\textsubscript\text{CO}_2 would then be expected to symmetrically affect the CO\textsubscript2 availability in both the bicarbonate secretory and bicarbonate absorbing cell.

Alternatively, changes in P\textsubscript\text{CO}_2 availability may not, in fact, influence either unidirectional HCO\textsubscript3\textsuperscript- transport flux. Studies have been performed in the turtle bladder which suggest that unidirectional H\textsuperscript+ secretion is relatively unaffected by anisohydric changes in P\textsubscript\text{CO}_2 over a broad range of values. Schwartz (32) measured proton secretion (J\textsubscriptH+) by reverse short circuit current in ouabain-treated turtle bladders under conditions of anisohy-
and Cl\textsuperscript- concentration gradients have a much more striking effect on net HCO\textsubscript{3}\textsuperscript{-} transport than do changes in peritubular pH.

The morphologic studies of Verlander et al. (17) suggest that proton secretion by the rat CCT is stimulated by in vivo respiratory acidosis. These investigators describe two subpopulations of intercalated cells in the rat CCT: a light form with prominent apical microprojections and a dark form with short and sparse apical projections. The light form displayed extensive proliferation of the apical membrane during respiratory acidosis, similar to that observed by Madsen and Tisher (16) in the outer medullary collecting duct of the rat during respiratory acidosis. These changes are consistent with increased insertion of proton pumps on the apical membrane of these cells (16). The authors suggest that the light intercalated cell is responsible for proton secretion (i.e., HCO\textsubscript{3}\textsuperscript{-} reabsorption) and the dark intercalated cell is responsible for HCO\textsubscript{3}\textsuperscript{-} secretion. Inasmuch as respiratory acidosis only changed one cell subpopulation, the authors suggest that respiratory acidosis stimulates net HCO\textsubscript{3}\textsuperscript{-} absorption in the CCT, enhancing urinary acidification. The previously cited studies of Schwartz and Al-Awqati (18) in the isolated perfused CCT also suggest a role for hypercapnea as a stimulus for increased insertion of proton pumps on the apical cell membrane of a subpopulation of intercalated cells in the CCT.

There are several possible explanations for the apparent discrepancy between our findings and these studies. The studies of Verlander, Madsen, and Tisher were on animals with 4 h of in vivo respiratory acidosis. The time of hypercapnea or any one of several other in vivo perturbations, such as altered catecholamines, or changes in the luminal fluid delivered to the CCT, could be an additional important variable.

The studies of Schwartz and Al-Awqati (18) examined simultaneous luminal and bath isohydric increases in PCO\textsubscript{2}. We examined only peritubular changes in these studies so no direct comparison can be made between the two studies. Small changes in net HCO\textsubscript{3}\textsuperscript{-} transport might go undetected in our study yet be associated with impressive changes in cell morphology. Even if this were the case one could still conclude that the effects of peritubular HCO\textsubscript{3}\textsuperscript{-} and Cl\textsuperscript- concentration are certainly predominant over those of either peritubular pH or PCO\textsubscript{2}. It is also possible that these morphologic changes may, in fact, not be associated with enhanced net urinary acidification by the CCT.

We would like to stress that these studies examined only net HCO\textsubscript{3}\textsuperscript{-} transport in cortical collecting tubules. In each of the protocols it is impossible to ascribe any significant change in net HCO\textsubscript{3}\textsuperscript{-} transport to changes in unidirectional proton secretion or HCO\textsubscript{3}\textsuperscript{-} secretion. Similarly, in protocols in which no effect on net HCO\textsubscript{3}\textsuperscript{-} transport was observed it is possible that significant but cancelling effects on unidirectional H\textsuperscript{+} secretion and HCO\textsubscript{3}\textsuperscript{-} secretion occurred.

In conclusion the current studies demonstrate that acute in vitro changes in ambient PCO\textsubscript{2} within the physiologic range do not affect net HCO\textsubscript{3}\textsuperscript{-} transport in the CCT. Conversely, alterations in peritubular HCO\textsubscript{3}\textsuperscript{-} and Cl\textsuperscript- concentration influence net transcellular HCO\textsubscript{3}\textsuperscript{-} transport. The effect of acute changes in peritubular HCO\textsubscript{3}\textsuperscript{-} concentration on J\textsubscript{VCO} in the CCT is independent of pH. From these findings we would suggest that blood pH is not the prime determinant of net urinary acidification by the CCT. It is unclear whether acute in vivo alterations in ambient PCO\textsubscript{2} affect net acidification of the urine by the CCT. Our studies would suggest that if PCO\textsubscript{2} does influence the rate of acidification by the CCT, the effect is either indirect, or small, when compared to the effect of altered peritubular anion concentration.

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