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 (P_{CO_2}), and HCO_3^- concentration on net HCO_3^- transport was examined in in vitro perfused cortical collecting tubules (CCTs) from unpretreated New Zealand white rabbits. Lowering peritubular HCO_3^- concentration and pH by reciprocal replacement of HCO_3^- with Cl^-, significantly stimulated net HCO_3^- absorption. Lowering peritubular HCO_3^- concentration and pH, by substitution of HCO_3^- with gluconate, while keeping Cl^- concentration constant, also stimulated net HCO_3^- absorption. Raising peritubular HCO_3^- concentration and pH, by reciprocal replacement of Cl^- with HCO_3^-, inhibited net HCO_3^- absorption (or stimulated net HCO_3^- secretion). When the tubule was cooled, raising peritubular HCO_3^- concentration had no effect on net HCO_3^- transport, suggesting these results are not due to the passive flux of HCO_3^- down its concentration gradient.

The effect of changes in ambient P_{CO_2} on net HCO_3^- transport were also studied. Increasing the ambient P_{CO_2} from 40 mmHg to either 80 or 120 mmHg, allowing pH to fall, had no effect on net HCO_3^- transport. Similarly, lowering ambient P_{CO_2} to 14 mmHg had no effect on net HCO_3^- transport. Simultaneously increasing peritubular HCO_3^- concentration and P_{CO_2}, without accompanying changes in peritubular pH, i.e., isohydric changes, stimulated net HCO_3^- secretion to the same degree as nonisohydric increases in peritubular HCO_3^- concentration. Likewise, isohydric lowering of peritubular HCO_3^- concentration and P_{CO_2} stimulated net HCO_3^- absorption.

We conclude that: (*a*) acute changes in peritubular HCO_3^- concentration regulate acidification in the CCT and these effects are mediated by a transcellular process; (*b*) acute changes in ambient P_{CO_2} within the physiologic range have no effect on HCO_3^- 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)[1] can display either net HCO_3^- absorption or secretion (4–9). Thus, the CCT may participate in the final acidification or alkalinization of the urine. The parameters modulating net HCO_3^- transport in the CCT have been only partially characterized. Isolated perfused tubule studies demonstrate that the direction of net HCO_3^- 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 augmented HCO_3^- absorption (4, 5, 7, 8), whereas tubules harvested from animals with chronic metabolic alkalosis display enhanced HCO_3^- secretion (4, 5, 9, 10). Thus, transport of HCO_3^- 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 HCO_3^- 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 CO_2 in the regulation of CCT HCO_3^- 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 HCO_3^- 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 acidic compartments with fluorescent probes, demonstrates augmented apical exocytosis in response to isohydric increases in ambient P_{CO_2}. However, no measurements of HCO_3^- flux or proton secretion were made under conditions that examined isolated changes of P_{CO_2} 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 HCO_3^- via apical cell membrane Cl–HCO_3^- exchange (19, 20). As noted above, in vitro perfused CCTs can secrete and reabsorb HCO_3^-. It is likely that both processes occur simultaneously and net HCO_3 transport is the sum of these two unidirectional fluxes.

To date, net HCO_3^- 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

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

HCO_3^- transport in the rat CCT (22). Direct acute effects of ambient acid-base conditions on net HCO_3^- 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 HCO_3^- concentration, pH, and P_{CO_2}, on net HCO_3^- 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 HCO_3^- concentration but not peritubular pH or P_{CO_2} which influences net HCO_3^- 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 between 0.5 and 2.0 nl/mm · min. The perfusate was collected in a constriction 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 (J_V), and then two to four total CO_2 flux (J_{TCO_2}) determinations were made. After determination of control period J_V and J_{TCO_2}, the bath was changed to one of 10 different experimental solutions. Another J_V determination was made, lasting 10–15 min. Then two to four additional determinations of J_{TCO_2} 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 P_{CO_2} (25) when a change in bath P_{CO_2} 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, $NaHCO_3$ 25, Na acetate 10, $NaHPO_4$ 2.3, $CaCL_2$ 1.8, $MgSO_4$ 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% O_2/5% CO_2 gas mixture to achieve a pH of 7.40.

The effects of ten different experimental conditions on J_V and J_{TCO_2} were examined. Their composition is shown in Table I. These solutions were designed to examine the effects of three different maneuvers on CCT HCO_3^- transport: (*a*) changing peritubular HCO_3^- concentration, (*b*) changing ambient P_{CO_2}, (*c*) isohydric changes in HCO_3^- concentration and P_{CO_2}.

Group 1: effect of peritubular HCO_3^- concentration. The tubule was equilibrated in the 25 mM HCO_3^- control bath. Control measurements were made, and then the bath was changed to either the 5, 10, or 50 mM HCO_3^--containing bath. Changes in peritubular HCO_3^- concentration were achieved by reciprocally changing peritubular Cl^- concentration except in the 10 mM HCO_3^- experiment where the peritubular HCO_3^- concentration was lowered by replacement with gluconate. This latter group of experiments was designed to measure the effect of peritubular HCO_3^- concentration in the absence of transtubular Cl^- concentration gradients.

The contribution of passive movement of HCO_3^- down a concentration gradient across the CCT was also examined. In this study the control solutions were symmetrical 25 mM HCO_3^--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 perfusate Cl^- concentration were 90 mM. This allowed us to impose a 25 mM bath to lumen HCO_3^- gradient without imposing any Cl^- gradient. In this protocol we first measured J_{TCO_2} at 37°C. The bath was then cooled to 20°C using a water-jacketed bath line. J_{TCO_2} was again measured. Finally, the bath was changed to a 50 mM HCO_3^-, P_{CO_2} 40, pH 7.70 solution identical to the metabolic alkalosis bath but at 20°C. J_{TCO_2} 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 P_{CO_2}. In addition to the control P_{CO_2} of 40 mmHg, we varied ambient P_{CO_2} from values as low as 8 mmHg up to 120 mmHg.[2] Ambient pH was allowed to vary.

Group 3: isohydric changes in ambient P_{CO_2} and HCO_3^- concentration. These studies examined the effect of changing the P_{CO_2} and HCO_3^- con-

Table I. Composition of Solutions

Solutions	pH	P_{CO_2}	HCO_3^-	Cl^-	Gluconate
		mM	*mM*	*mM*	*mM*
Control	7.40	40	25	115	
Metabolic acidosis	6.96	40	5	135	
Metabolic alkalosis	7.70	40	50	90	
Anion gap acidosis	7.05	40	10	115	15
Cooling control	7.4	40	25	90	25
Moderate respiratory acidosis	7.10	80	25	115	
Severe respiratory acidosis	6.90	120	25	115	
Moderate respiratory alkalosis	7.80	14	25	115	
Severe respiratory alkalosis	8.1	8	25	115	
Isohydric elevation CO_2 and HCO_3^-	7.4	80	50	90	
Isohydric decrease CO_2 and HCO_3^-	7.4	18	10	130	

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, Mg^{2+} 1, acetate$^-$ 10, glucose 8, alanine 5, HPO_4^{2-} 2.3, and Ca^{2+} 1.8. The bath also contained 5% vol/vol fetal calf serum while the perfusate contained exhaustively dialyzed inulin.

2. Previous studies (23) have verified a close correlation between syringe bath pH and the pH of the bath reaching the tubule at P_{CO_2} tensions of 15–74. In preliminary studies using pH microelectrodes in the bath we also verified that the pH of the 120 mmHg P_{CO_2} bath reaching the tubule (at 37°C) was at least as acid as that in the syringe (bath pH 0.07 lower than syringe). This shows that there is no detectable loss of CO_2 between the syringe and the tubule.

centration simultaneously so that bath pH did not vary. In this group bath HCO_3^- concentration was changed by reciprocally altering Cl^- concentration. In the control period the bath, as usual, contained 25 mM HCO_3^-, P_{CO_2} 40 mmHg, pH 7.40. The experimental solutions examined either an isohydric increase in HCO_3^- concentration (50 mM, P_{CO_2} 80, pH 7.40) or an isohydric decrease in HCO_3^- concentration (10 mM, P_{CO_2} 18 mmHg, pH 7.40).

Microassays

Net volume flux (J_V) was determined from changes in the [^{3}H]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 J_V greater than±0.05 nl/mm · min was discarded.

J_{TCO_2} was determined by microcalorimetry (28) and calculated according to the equation: $J_{TCO_2} = \{[CO_2]_{in} - [CO_2]_{out}\} \cdot V_0/\text{min} \cdot \text{l/length}$ (mm). V_0/min equals the rate the perfusate was collected. This equation assumes negligible change in HCO_3^- concentration due to water flux (as excluded by the J_V determinations). Samples were injected immediately after collection into concentrated sulfuric acid in the picapnotherm chamber (University of California Research Dept.). This value was then compared to a paired injection of the 25 mM Na_2CO_3 standard. The J_{TCO_2} 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 perfusate total CO_2 (TCO_2) and [^{3}H]inulin content were then assayed using the same constant volume pipette as used during the experiment. The perfusate TCO_2 was measured two to four times and the values averaged. The measured TCO_2 was the value used for $[CO_2]_{in}$. 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 TCO_2 collection per period is 0.8 mM/$\sqrt{3.26}$ (i.e., standard error of the mean) or 0.44 mM (29). Thus, we could reliably determine differences of 0.88 mM TCO_2 between experiment periods.

In those experiments where ambient P_{CO_2} was altered, the TCO_2 of the collected perfusate was corrected for the contribution that the change in P_{CO_2} would theoretically make in measured total CO_2. Thus if the P_{CO_2} of the bath was raised from 40 to 80 mmHg between control and experimental periods the TCO_2 of the collected perfusate would be expected to increase 0.03 · P_{CO_2} (mmHg) or, in this case, 1.2 mM. Therefore, 1.2 mM was subtracted from the measured TCO_2 in each of the experimental period collections. Because there is some loss of CO_2 into the oil in the collection pipette this correction tends to slightly overestimate increases in HCO_3^- absorption and underestimate increases in HCO_3^- secretion.[3]

In studies with two periods the J_{TCO_2} 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.

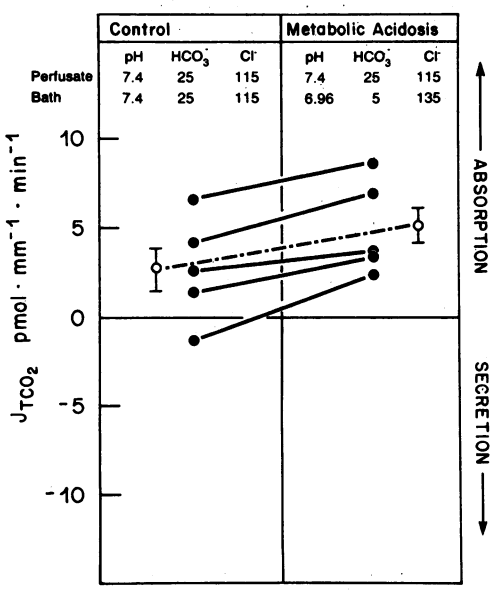

Figure 1. In vitro peritubular metabolic acidosis increases net HCO_3^- absorption in the CCT. Individual tubules (n = 5) (●). Mean J_{TCO_2}±SE in pmol/mm · min (○). Control 2.8±1.3 vs. experimental 5.1±1.2, $P < 0.02$.

Results

Effect of changing peritubular HCO_3^- concentration. The peritubular environment during acute metabolic acidosis was simulated by lowering peritubular pH from 7.4 to 6.96 and the bath HCO_3^- concentration from 25 to 5 mM. Bath HCO_3^- 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 transepithelial 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 J_{TCO_2} 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 I). Thus lowering the peritubular HCO_3^- and pH significantly stimulates HCO_3^- absorption in the CCT.

We also attempted to simulate the peritubular environment during acute metabolic alkalosis by raising the peritubular HCO_3^- concentration from 25 to 50 mM, raising pH from 7.4 to 7.7. Control and experimental period perfusion rates were

3. Preliminary experiments documented loss of CO_2 into the oil phase with time; previous work (26) has shown that the correction for P_{CO_2} between 14 and 40 mmHg is correct; that the correction for P_{CO_2} between 40 and 80 mmHg is off by only 0.4 mM; and that the use of CO_2-equilibrated oil retards the loss of CO_2 from the collected perfusate. In the group of experiments examining the effect of 120 mmHg P_{CO_2} we used mineral oil equilibrated with 5% and 15% CO_2 behind the collected perfusate during control and experimental periods, respectively. The increase in TCO_2 owing to the increase in dissolved CO_2 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 HCO_3^- in this segment (8, 9) and rapid perfusion makes any potential residual active transport of HCO_3^- in this segment, undetectable (4). Cooling could not be used to inhibit transport in this case because it would change the solubility of CO_2 in oil and aqueous phase. We documented the inhibition of active transport by this technique by comparing the TCO_2 collected from a tubule treated in this manner (in a P_{CO_2} of 40 mmHg) and the TCO_2 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 TCO_2 was then measured. The difference in TCO_2 measured when the acetazolamide-treated tubule was perfused with an ambient P_{CO_2} of 120 vs. 40 mmHg, averaged 2.0 mM. This number was used to correct for the contribution that difference in dissolved CO_2 makes to the change in TCO_2 collected during each period. Correction of the measured collected TCO_2, for the theoretical contribution that titration of HCO_3^- by luminal phosphate makes to TCO_2, does not change the statistical significance of our data.

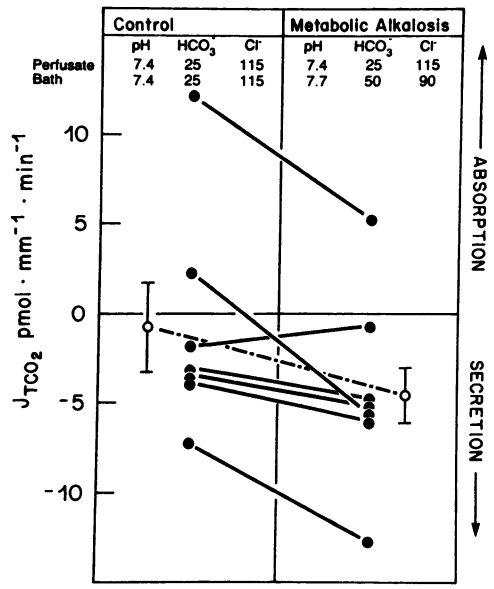

Figure 2. In vitro peritubular metabolic alkalosis inhibits net HCO_3^- absorption in the CCT. Individual tubules (n = 7) (●). Mean J_{TCO_2}±SE in pmol/mm · min (○). Control −0.7±2.4 vs. experimental −4.5±1.4, $P < 0.05$.

1.92±0.34 and 1.98±0.23 nl/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 HCO_3^- secretion was stimulated or absorption inhibited by raising peritubular HCO_3^- concentration (control −0.7±2.4 vs. experimental −4.5±1.4 pmol/mm · min, $P < 0.05$). Thus metabolic alkalosis stimulates net HCO_3^- secretion in the CCT.

Because in both of these experiments transtubular Cl^- as well as HCO_3^- concentration gradients existed, we examined the effect of lowering the bath HCO_3^- concentration without simultaneously imposing a Cl^- gradient (Fig. 3, Table II). Peritubular HCO_3^- 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 HCO_3^- 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 J_{TCO_2} increased from 2.12±1.22 to 4.38±0.86 pmol/mm · min ($P < 0.005$). The change in J_{TCO_2} 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 HCO_3^- and pH can modulate HCO_3^- 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 HCO_3^- down its concentration gradient could contribute to our results, we cooled the tubule to 20°C and then measured net HCO_3^- transport before and after imposing a 25 mM HCO_3^- gradient from bath to lumen (Fig. 4, Table II). No transepithelial Cl^- gradient existed in these experiments. In the first period net HCO_3^- 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 J_{TCO_2} 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 J_{TCO_2} fell to −0.10±0.31 pmol/mm · min, a value not statistically different from 0. The peritubular HCO_3^- 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 J_{TCO_2} was unchanged (0.20±0.14 pmol/mm · min, $P > 0.30$ compared with previous period). Thus, there is no change in J_{TCO_2} when a 25 mM bath to lumen HCO_3^- gradient is imposed on CCTs in which active transport is inhibited by cooling to 20°C.

Effect of changing ambient P_{CO_2}. In experiments designed to simulate acute respiratory acid-base disorders, the bath P_{CO_2} 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 P_{CO_2} 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 J_{TCO_2} 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 P_{CO_2} and lowering the pH had no effect on net HCO_3^- transport.

Micropuncture studies in the rat have shown that during acute in vivo respiratory acidosis renal cortical P_{CO_2} may be as much as 50 mmHg greater than the arterial P_{CO_2} (30). Renal cortical P_{CO_2} in the rabbit has also been found to be roughly 20 mmHg higher than arterial P_{CO_2} under eucapneic conditions (31). Furthermore, a variety of studies suggest that acute in vivo respiratory acidosis stimulates HCO_3^- absorption in the distal nephron and CCT in particular (3, 17).

It is possible that doubling the bath P_{CO_2} might be insufficient to evoke a measurable increase in HCO_3^- absorption. We therefore examined the effect of raising bath P_{CO_2} from 40 to 120 mmHg, on net T_{CO_2} 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 it was the reverse. 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 P_{CO_2} 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 P_{CO_2} bath

Table II. Microperfusion Data

		n	Perfusion rate	Perfusate TCO_2	Collected TCO_2	J_{TCO_2}
			nl/mm · min	*mM*	*mM*	*pmol/mm · min*
Metabolic acidosis	C	5	1.72±0.10	23.8±0.35	22.0±0.75	2.8±1.3] *
	E		1.53±0.07		20.4±0.78	5.1±1.2] *
Metabolic alkalosis	C	7	1.92±0.34	23.3±0.41	24.1±0.97	−0.7±2.4] *
	E		1.98±0.23		25.9±0.94	−4.5±1.4] *
Anion gap acidosis	C	10	0.85±0.08	24.8±1.05	21.7±1.36	2.21±0.7] §
	E		0.84±0.08		18.7±1.15	4.38±0.7] §
21°C high HCO_3^-	C	4	0.59±0.09	25.2±0.36	23.0±0.71	1.32±0.4
	E		0.56±0.06		25.1±0.45	−0.10±0.3
	R		0.59±0.03		25.2±0.33	0.20±0.1
Moderate respiratory acidosis	C	6	1.06±0.08	24.4±0.20	22.8±0.74	1.44±0.7
	E		1.06±0.04		22.3±1.15	2.36±1.5
Severe respiratory acidosis	C	5	0.90±0.17	25.5±0.34	25.7±0.95	−0.96±1.12
	E		0.87±0.17		25.5±1.88	−0.86±1.38
Moderate respiratory alkalosis	C	7	1.07±0.10	23.9±0.33	21.0±0.96	3.11±1.23
	E		1.10±0.12		20.9±1.00	3.27±1.34
Severe respiratory alkalosis	C	3	0.80±0.11	23.8±0.33	18.1±1.99	3.91±0.71]] *
	E		0.80±0.10		23.2±0.53	0.30±0.21] *]
	R		0.73±0.05		20.9±0.97	2.01±0.55] *
Isohydric increase HCO_3^-	C	5	0.93±0.08	24.3±0.16	27.4±0.89	−2.69±0.83] ‡
	E		1.18±0.09] *		31.0±1.72	−6.83±1.31] §
	R		0.92±0.05] *		27.2±0.97	−2.47±0.79] §
Isohydric decrease HCO_3^-	C	4	0.66±0.13	25.9±0.23	25.9±0.79	0.21±0.57] §] §
	E		0.65±0.14		20.0±1.45	3.39±0.76] §
	R		0.67±0.08		22.0±1.16	2.59±0.72]

C, control period; E, experimental period; R, recovery period. Values given as mean±SE. J_{TCO_2} is net TCO_2 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 P_{CO_2} to 80 mmHg, no significant change in J_{TCO_2} was observed between the periods of eucapnea and hypercapnea to 120 mmHg (Fig. 6, Table II). The mean J_{TCO_2} 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 hypercapnea on J_{TCO_2} also failed to reveal a significant effect of this maneuver ($P > 0.40$). Thus, acidosis due to hypercapnea does not stimulate HCO_3^- absorption in the isolated perfused CCT.

Moderate in vitro respiratory alkalosis was also studied (Fig. 7, Table II). The ambient P_{CO_2} 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 J_{TCO_2} 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 HCO_3^- transport in the CCT.

Because CO_2 has been shown to be important to HCO_3^- 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 HCO_3^- transport. The bath P_{CO_2} was acutely lowered to 8 mmHg (Fig. 8, Table II).

During acute in vivo respiratory alkalosis in the rat, renal cortical P_{CO_2} was directly measured as 25 mmHg at a time when arterial P_{CO_2} was 15 mmHg (30). Even if an animal could be hyperventilated to an arterial P_{CO_2} of 8 mmHg, the renal cortical P_{CO_2} would not be this low. This degree of hypocapnea is thus well outside the physiologic range.

Raising the ambient pH and lowering the P_{CO_2} to 8 mmHg depolarized the transepithelial PD from −30.0±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 0.73±0.05 nl/mm · min, $P > 0.48$ in all comparisons). In three tubules, with

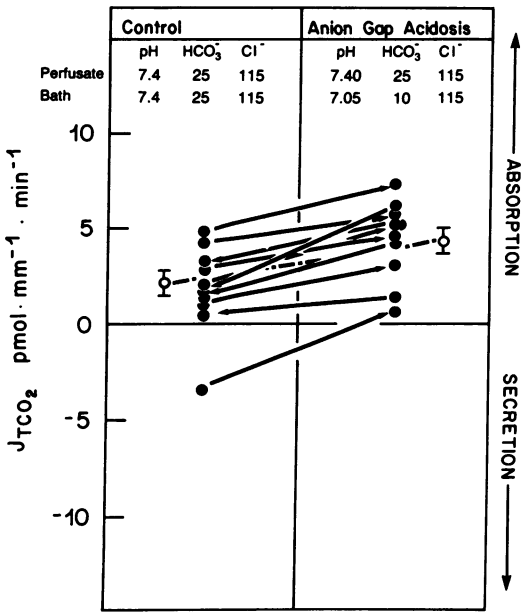

Figure 3. In vitro peritubular anion gap metabolic acidosis stimulates net HCO_3^- absorption in the CCT. Peritubular [HCO_3^-] was lowered by replacing HCO_3^- with gluconate. Individual tubules (n = 10) (●). The arrows indicate the sequence of bath change. Mean J_{TCO_2}±SE in pmol/mm · min (○). Control 2.21±0.74 vs. experimental 4.38±0.66, $P < 0.0005$.

a mean length of 2.15±0.05 mm, lowering the P_{CO_2} to 8 mmHg significantly suppressed HCO_3^- absorption 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 HCO_3^- absorption to

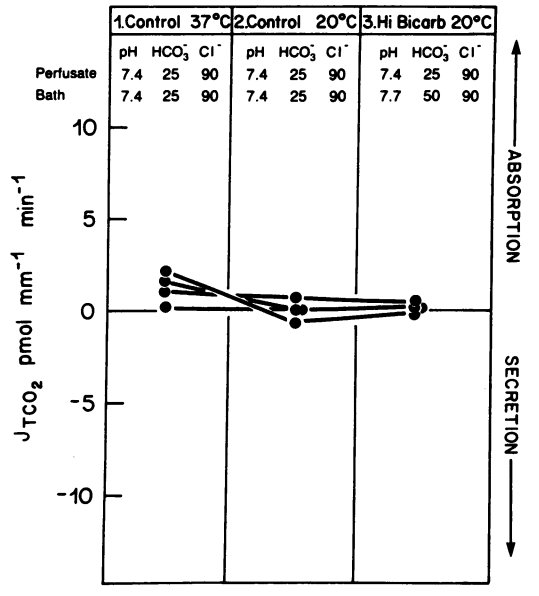

Figure 4. Raising bath [HCO_3^-] does not affect net HCO_3^- transport in tubules cooled to 20°C. Bath [HCO_3^-] was increased without changing [Cl^-] by substituting it for the 25 mM gluconate present in control bath. Individual tubules (n = 4) (●). Mean J_{TCO_2}±SE in pmol/mm · min. Period 1, 1.32±0.40; 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 $P < 0.70$.

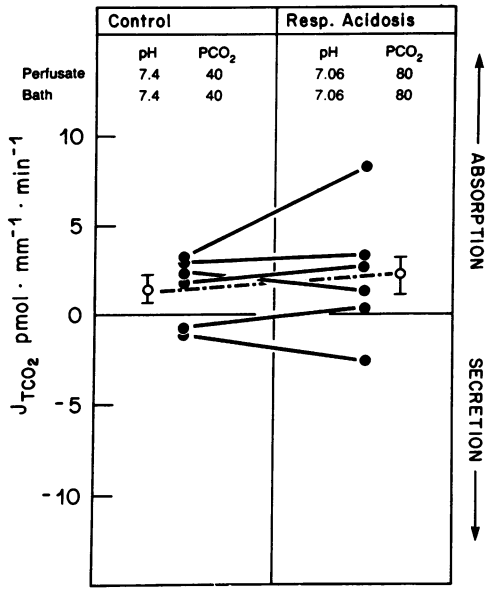

Figure 5. In vitro moderate respiratory acidosis (P_{CO_2} = 80 mmHg) has no effect on net HCO_3^- transport in the CCT. Individual tubules (n = 6) (●). Mean J_{TCO_2}±SE in pmol/mm · min (○). Control 1.44±0.74 vs. experimental 2.36±1.50, $P > 0.40$.

2.01±0.55 pmol/mm · min. This recovery value was statistically different from the control HCO_3^- flux ($P < 0.05$). Thus, although respiratory acidosis and moderate respiratory alkalosis do not measurably affect HCO_3^- transport in the CCT, severe reduction of the P_{CO_2} does result in inhibition of HCO_3^- transport.

Effects of isohydric changes in P_{CO_2} and HCO_3^- concentration. By simultaneously changing bath HCO_3^- concentration and P_{CO_2} we were able to vary peritubular HCO_3^- concentration without

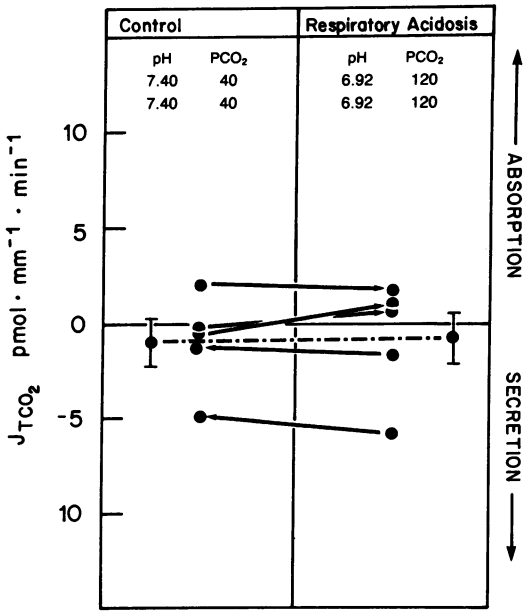

Figure 6. In vitro severe respiratory acidosis (P_{CO_2} = 120 mmHg) has no effect on net HCO_3^- transport in the CCT. Individual tubules (n = 5) (●). Mean J_{TCO_2}±SE in pmol/mm · min (○). Control −0.96±1.12 vs. experimental −0.86±1.38, $P < 0.80$.

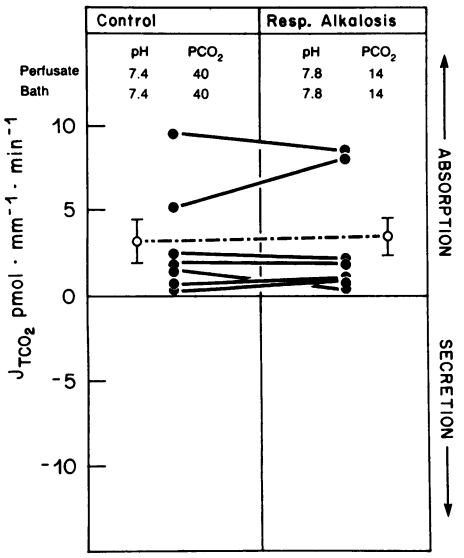

Figure 7. In vitro moderate respiratory alkalosis (P_{CO_2} = 14 mmHg) has no effect on net HCO_3^- transport in the CCT. Individual tubules (n = 7) (●). Mean J_{TCO_2}±SE in pmol/mm · min (○). Control 3.11±1.23 vs. experimental 3.26±1.34, $P > 0.50$.

varying pH. This maneuver enables one to determine whether the modulation of net HCO_3^- transport by changes in peritubular HCO_3^- and/or 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 HCO_3^- concentration replacing Cl^- milliequivalent for milliequivalent. The peritubular pH was maintained constant at 7.40 by simultaneously doubling the ambient P_{CO_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±0.08, experimental 1.18±0.09, recovery 0.92±0.05 nl/mm · min). In five tubules averaging 2.17±0.14 mm, the PD in the control period averaged −21.7±4.8 mV vs. −20.8±3.9 mV ($P > 0.70$, NS) during the experimental period. Doubling the peritubular HCO_3^- concentration without changing pH stimulated HCO_3^- secretion in every case (Fig. 9, Table II). The mean J_{TCO_2} in the control period was −2.69±0.83 pmol/mm · min and increased to −6.83±1.31 pmol/mm · min during the experimental period. This stimulation was fully reversible by returning to the control bath. The change in net HCO_3^- flux observed with the isohydric increase in peritubular HCO_3^- concentration was comparable to that observed with nonisohydric changes in bath HCO_3^- concentration (Δ = −4.14 vs. −3.80 pmol/mm · min). Thus, raising bath HCO_3^- stimulates net HCO_3^- secretion whether the peritubular pH increases or remains constant.

The peritubular HCO_3^- concentration was also isohydrically acutely lowered by replacing HCO_3^- with Cl^- milliequivalent for milliequivalent. The ambient P_{CO_2} was lowered to a value within a range of P_{CO_2} tensions shown, by earlier experiments, not to independently alter net HCO_3^- transport in the CCT (i.e., ≧14 mmHg). The perfusion rate was unchanged (control 0.66±0.13, experimental 0.65±0.14, recovery 0.67±0.08 nl/mm · min, P ≧ 0.84 in all comparisons). In four tubules, averaging 2.08±0.17 mm in length, the PD in the control period was −13.3±3.9 mV vs. a mean PD of −15.0±6.9 mV during the experimental period ($P > 0.60$, NS). Isohydrically lowering bath HCO_3^- concentration to 10 mM and the P_{CO_2} to 18 mmHg significantly stimulated net HCO_3^- absorption in four of four tubules from a mean J_{TCO_2} of 0.21±0.57 to 3.39±0.76 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 HCO_3^- absorption to 2.59±0.72 pmol/mm · min ($P < 0.08$). This

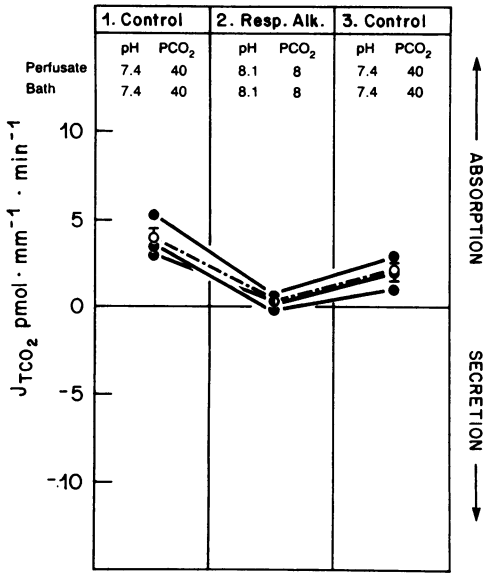

Figure 8. In vitro severe respiratory alkalosis (P_{CO_2} = 8 mmHg) inhibits net HCO_3^- absorption in the CCT. Individual tubules (n = 3) (●). Mean J_{TCO_2}±SE in pmol/mm · min (○). Period 1, 3.91±0.71; period 2, 0.30±0.21; period 3, 2.01±0.55; period 1 vs. 2 $P < 0.01$, period 2 vs. 3 $P < 0.05$, period 1 vs. 3 $P > 0.05$.

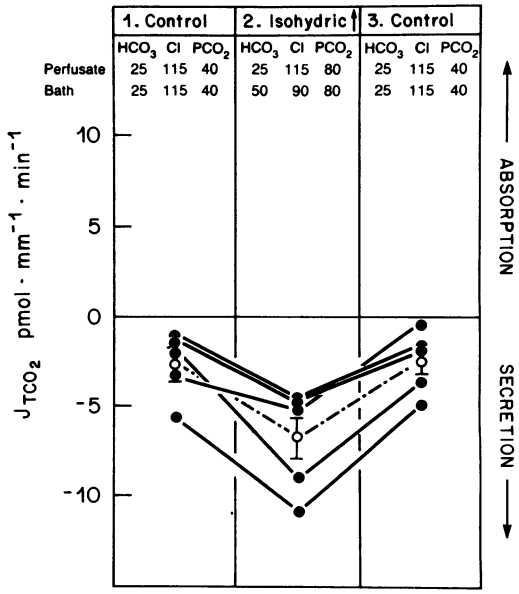

Figure 9. Isohydric increase in bath [HCO_3^-] and P_{CO_2} stimulates net HCO_3^- secretion in the CCT. Individual tubules (n = 5) (●). Mean J_{TCO_2}±SE in pmol/mm · min (○). Period 1, −2.69±0.83; period 2, −6.83±1.31; period 3, −2.41±0.79, period 1 vs. 2 $P < 0.01$, period 2 vs. 3 $P < 0.001$, period 1 vs. 3 $P > 0.50$.

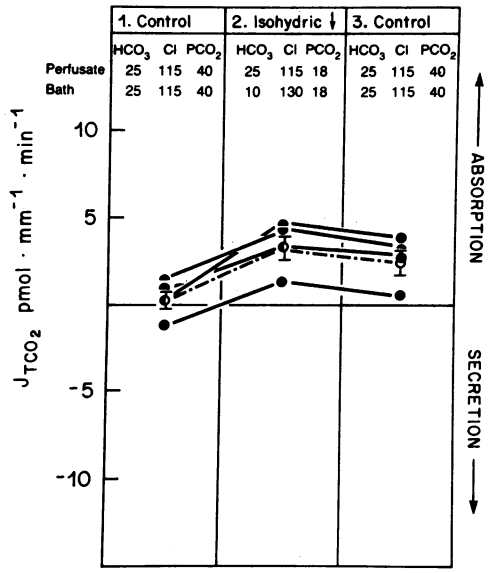

Figure 10. Isohydric decrease in bath [HCO_3^-] and P_{CO_2} stimulates net HCO_3^- absorption in the CCT. Individual tubules ($n = 4$) (●). Mean J_{TCO_2}±SE in pmol/mm · min (○). Period 1, −0.21±0.57; period 2, 3.39±0.76; period 3, 2.59±0.72; period 1 vs. 2 $P < 0.001$, period 2 vs. 3 $P < 0.08$, period 1 vs. 3 $P < 0.001$.

recovery was only partial since J_{TCO_2} was not significantly different from experimental J_{TCO_2} 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 P_{CO_2} on net HCO_3^- transport in the CCT. Lowering the peritubular HCO_3^- and pH stimulated net HCO_3^- absorption whereas raising the peritubular HCO_3^- and pH stimulated 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 P_{CO_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 P_{CO_2} was lowered to ~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 P_{CO_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^- concentration and HCO_3^- concentration. Isolated changes in peritubular HCO_3^- concentration would be expected to change transcellular HCO_3^- flux if the above model is correct. Changing the HCO_3^- concentration would alter the driving force across the Cl^-/HCO_3^- exchanger in the proton secretory cell and might alter basolateral proton pumping in the HCO_3^- secretory cell by changing the peritubular pH and the electrochemical driving force for H^+ pumping. Because we demonstrate in these studies that lowering the peritubular HCO_3^- concentration without changing peritubular Cl^- concentration stimulates net HCO_3^- absorption, our data verifies that Cl^- concentration is not the sole factor modulating HCO_3^- 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_3^- flux down its concentration gradient. Active HCO_3^- transport was inhibited by cooling the tubule to 20°C. A HCO_3^- concentration gradient was then imposed. The results show no change in J_{TCO_2} when cooled tubules are exposed to changes in peritubular HCO_3^- concentration. Under these conditions, in CCTs from unpretreated 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°C. They could not detect any HCO_3^- conductance. Paracellular ionic movement is conductive in nature so the absence of HCO_3^- conductance in this segment argues strongly against paracellular bicarbonate movement. In accord with these findings are the experiments in which P_{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_{TCO_2}. This is in agreement with the lack of conductive movement of HCO_3^- in this segment. The lack of an effect of HCO_3^- concentration gradients in cooled CCTs also argues against passive paracellular movement of HCO_3^- in the CCT. Hence changes in peritubular HCO_3^- concentration modulate a transcellular transport process.

The next series of experiments examined the effects of altering ambient P_{CO_2} within the physiologic range. We found no effect of changing P_{CO_2}, over a range from 14 to 120 mmHg, on net HCO_3^- transport in the CCT. There are several possible explanations for this lack of an effect of changes in P_{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_{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_2 and carbonic anhydrase for the formation of HCO_3^- from intracellular OH^- ion and CO_2 as outlined previously. The experimental changes in P_{CO_2} would then be expected to symmetrically affect the CO_2 availability in both the bicarbonate secretory and bicarbonate absorbing cell.

Alternatively, changes in P_{CO_2} availability may not, in fact, influence either unidirectional HCO_3^- transport flux. Studies have been performed in the turtle bladder which suggest that unidirectional H^+ secretion is relatively unaffected by anisohydric changes in P_{CO_2} over a broad range of values. Schwartz (32) measured proton secretion (J_{H^+}) by reverse short circuit current in ouabain-treated turtle bladders under conditions of anisohydric increases in serosal P_{CO_2}. Although there was a linear increase in reverse short circuit current with increases in P_{CO_2} between 0 and 4.5%, from 4.5 to 20% CO_2 no further increase in reverse short circuit current was observed. Cohen and Steinmetz (33) also found that as P_{CO_2} was increased a plateau in J_{H^+} was achieved. The P_{CO_2} at which this plateau was reached, was variable and directly proportional to the serosal HCO_3^- concentration. This suggests that cell pH rather than cell P_{CO_2} may be the critical variable. At low P_{CO_2} values, these investigators also observed a linear increase in J_{H^+} with increasing P_{CO_2}. The slope of this linear increase, decreased as serosal HCO_3^- concentration was increased. This is consistent with a relationship between cell pH and J_{H^+} in the alkaline range. Cell pH was measured with DMO. Half-maximal proton secretory rates occurred at an estimated intracellular $[H^+]$ of 25 nM or when cell pH is about 7.60. Under control conditions (ambient P_{CO_2} of 5% and 20 mM HCO_3^-) cell pH was estimated as 7.28 or a $[H^+]$ of 52 nM. Therefore, the cell is relatively acid under control conditions and control J_{H^+} is already at 80% of maximal rates. Apparently cell pH becomes a critical determinant of J_{H^+} only when the cell is very alkaline compared with control state. When cell $[H^+]$ increased from 52 to 186 nM there is only a 20% additional increase in J_{H^+}. In the turtle bladder changes in P_{CO_2} appear to have an affect on J_{H^+} only when they make the cell alkaline and do not greatly affect the J_{H^+} when raising the ambient P_{CO_2} causes cell pH to fall below 7.4. Similar studies, examining the relationship between serosal P_{CO_2} and unidirectional bicarbonate secretion have not been performed. Any relationship between serosal P_{CO_2} and pH on unidirectional bicarbonate secretion remains speculative.

The current studies found no change in net HCO_3^- absorption when P_{CO_2} was varied between 14 and 120 mmHg. Only when the ambient P_{CO_2} was lowered to less than 14 mmHg did net HCO_3^- transport fall. No increase in HCO_3^- absorption was found when the ambient P_{CO_2} was doubled or even tripled. These findings are similar to the previous studies cited in so far as a major effect of P_{CO_2} on HCO_3^- absorption occurs only in the hypocapneic end of the spectrum, i.e., when the cell would be expected to become alkaline. Near-maximal rates of HCO_3^- transport appear to be achieved at a lower ambient P_{CO_2} than is observed in the turtle bladder. There may be several reasons for these differences including (*a*) relative insensitivity of the assay (microcalorimetry in the CCT vs. electrically determined proton secretion in turtle bladder), (*b*) the measurement of net HCO_3^- flux vs. unidirectional proton secretion, (*c*) physiologic differences in the epithelium studied, such as different cell buffering power, different rates of metabolic CO_2 production, or differences in substrate affinity of H^+ or HCO_3^- transporters. Nevertheless, assuming that cell pH changes when ambient P_{CO_2} is changed, these studies suggest the same conclusion: cell pH is probably not a critical regulator of net acidification in the CCT outside of the alkaline range. Under physiologic conditions the regulation of CCT acidification may occur on the plateau portion of the J_{H^+}/cell pH curve where factors other than cell pH play a predominant role. This conclusion is further strengthened by the finding that the changes in net HCO_3^- transport induced by isohydric changes in peritubular HCO_3^- concentration are roughly the same as the effect of nonisohydric changes in peritubular HCO_3^- concentration. Isohydric changes in peritubular HCO_3^- would presumably change cell pH much less than nonisohydric changes (if at all), yet no discernable difference in ΔJ_{TCO_2} is seen between these two experimental groups. It seems that HCO_3^-

and Cl^- concentration gradients have a much more striking effect on net HCO_3^- 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_3^- reabsorption) and the dark intercalated cell is responsible for HCO_3^- secretion. Inasmuch as respiratory acidosis only changed one cell subpopulation, the authors suggest that respiratory acidosis stimulates net HCO_3^- 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 additional important variables.

The studies of Schwartz and Al-Awqati (18) examined simultaneous lumen and bath isohydric increases in P_{CO_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_3^- 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_3^- and Cl^- concentration are certainly predominant over those of either peritubular pH or P_{CO_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_3^- transport in cortical collecting tubules. In each of the protocols it is impossible to ascribe any significant change in net HCO_3^- transport to changes in unidirectional proton secretion or HCO_3^- secretion. Similarly, in protocols in which no effect on net HCO_3^- transport was observed it is possible that significant but cancelling effects on unidirectional H^+ secretion and HCO_3^- secretion occurred.

In conclusion the current studies demonstrate that acute in vitro changes in ambient P_{CO_2} within the physiologic range do not affect net HCO_3^- transport in the CCT. Conversely, alterations in peritubular HCO_3^- and Cl^- concentration influence net transcellular HCO_3^- transport. The effect of acute changes in peritubular HCO_3^- concentration on J_{TCO_2} 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 P_{CO_2} affect net acidification of the urine by the CCT. Our studies would suggest that if P_{CO_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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