Validation of the Difference in Urine and Blood Carbon Dioxide Tension During Bicarbonate Loading as an Index of Distal Nephron Acidification in Experimental Models of Distal Renal Tubular Acidosis

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Abstract

Recent classifications of the several pathophysiologic types of distal renal tubular acidosis (secretory, voltage dependent, and gradient) have been based on the response of acidification parameters to a series of provocative maneuvers in vivo and in vitro. A reduction in the difference in urine and blood CO_2 tension during bicarbonate loading (U-B pCO_2 gradient), a widely applied parameter, has been employed as an index of reduced distal nephron proton secretion. This study was designed to test the validity of the U-B pCO_2 gradient in a variety of experimental models of distal renal tubular acidosis by measuring and comparing disequilibrium pH (a direct technique to detect H^+ secretion in situ) with the pCO_2 in the papillary collecting duct of the rat in vivo during bicarbonate loading. Chronic amiloride, lithium chloride, and amphotericin-B administration, and the post-obstructed kidney models were employed. Amiloride resulted in an acidification defect which did not respond to sulfate infusion (urine pH = 6.15 ± 0.08), and was associated with an obliteration of the acid disequilibrium pH (-0.26 ± 0.05--0.08 ± 0.03) and reduction in papillary pCO_2 (116.9 ± 3.2-66.9 ± 2.5 mmHg). The defect induced by lithium administration responded to Na_2SO_4 (urine pH = 5.21 ± 0.06) but was similar to amiloride with respect to the observed reduction in disequilibrium pH (-0.04 ± 0.02) and pCO_2 (90.3 ± 3.0 mmHg). The post-obstructed kidney model was characterized by an abnormally alkaline urine pH unresponsive to sulfate (6.59 ± 0.06) and a reduction in disequilibrium pH ($+0.02\pm0.06$) and pCO_2 (77.6 ± 3.6 mmHg). Amphotericin-B resulted in a gradient defect as characterized by excretion of an acid urine after infusion of sodium sulfate (5.13 ± 0.06). Unlike other models, however, amphotericin-B was associated with a significant acid disequilibrium pH (-0.11 ± 0.05) and an appropriately elevated urine pCO_2 (119.8 ± 6.4 mmHg) which did not differ from the respective values in control rats. Thus, these findings support the use of the U-B pCO_2 as a reliable means of demonstrating impaired distal nephron proton secretion

in secretory and voltage-dependent forms of distal renal tubular acidosis (RTA) and supports the view that proton secretion is not impaired in gradient forms of distal RTA.

Introduction

Based on numerous observations in man and experimental animals, several distinctly different pathophysiologic mechanisms have been proposed to explain the inability to excrete a maximally acid urine in distal renal tubular acidosis (DRTA) (1–4).[1] The classical view that the disorder is primarily the result of an inability to maintain a pH gradient across the distal portion of the nephron (1) was based on the observation that bicarbonate reabsorptive capacity, and presumably hydrogen ion secretion, was intact during bicarbonate loading. In addition, titratable acid excretion could be increased during phosphate infusion in these patients (5). Therefore, it was suggested (1) that the failure of patients with classical DRTA to lower urine pH maximally during an acid challenge was the result of an inability to generate or maintain a maximum pH gradient across the distal nephron (gradient defect). However, primary impairment of the proton pump (pump or secretory defect) was suggested by the observations of Halperin and associates (6), since patients with classical DRTA failed to generate appropriately high values for urine pCO_2 during bicarbonate infusion (urine pH > 7.5). While the difference in urine and blood CO_2 tension during bicarbonate loading (U-B pCO_2 gradient) has been criticized on technical and physicochemical grounds (7, 8), studies in our laboratory have demonstrated that the U-B pCO_2 difference is an adequate qualitative index of distal nephron proton secretion in normal rats excreting highly alkaline urine (9).

However, as employed widely, a reduction in the U-B pCO_2 gradient has become synonymous with a reduction in distal nephron hydrogen ion secretion (2, 6). While it has been demonstrated that the increase in papillary collecting duct (CD) pCO_2 during bicarbonate loading in control rats is accompanied by an acid disequilibrium pH (pH_{Dq}) in this segment (9), a similar examination of hydrogen ion secretion in experimental models of DRTA has not been performed previously. Before the validity of U-B pCO_2 can be established,

1. *Abbreviations used in this paper:* BCD, base collecting duct; BW, body weight; CD, collecting duct; U-B pCO_2 gradient, difference in urine and blood CO_2 tension during bicarbonate loading; pH_{Dq}, disequilibrium pH; DRTA, distal renal tubular acidosis; pH_{Eq}, equilibrium pH; pH_{is}, in situ pH; RTA, renal tubular acidosis; TCD, tip collecting duct.

therefore, direct examination of terminal nephron hydrogen ion secretion concomitant with measurement of collecting duct pCO_2 in established models of DRTA appears to be necessary.

The purpose of this study was to measure and compare the magnitude of the pH_{Dq} and pCO_2 in the papillary CD in a wide variety of experimental models of DRTA during bicarbonate-loading in order to determine the reliability of the U-B pCO_2 gradient as an index of impaired distal nephron proton secretion.

Methods

Preparation of rats for micropuncture. Studies were performed after 100 mg/kg i.p. of inactin (BYK) anesthesia (Andrew Lockwood and Associates, East Lansing, MI) on young (70–125 g) male mutant Munich-Wistar rats (Simonsen Laboratories, Gilroy, CA or Harlen Sprague-Dawley, Indianapolis, IN). All rats were allowed free access to tap water and standard rat chow until the time of anesthesia. Surgical preparation of animals for papillary micropuncture was accomplished as reported previously (9, 10). All rats were maintained on a volume-regulated rodent ventilator (Harvard Apparatus, Inc., Millis, MA) and arterial blood gases were monitored frequently as described previously (9). For all groups, the inspired gas content was 40% O_2 (balance N_2). After jugular vein cannulation, surgical volume losses were routinely replaced by a volume of Ringer's bicarbonate (Na^+ = 140, Cl^- = 110, HCO_3^- = 25, K^+ = 5 meq/liter) equal to 1.5% of the rat's body weight (BW) over 15 min. An infusion at 1.0% BW/h was begun immediately thereafter. The left kidney was gently separated from the adrenal gland and peritoneal attachments, the ureter excised and the renal papilla exposed as reported previously (10). The kidney was then placed in a Lucite cup stabilized by 3% agar and continuously bathed with mineral oil equilibrated with 5% CO_2–95% O_2, maintained at 37°C, and illuminated with a small fiber optic light source. Urine was obtained from the right, nonexperimental kidney via the urinary bladder with PE-90 tubing into preweighed vials containing water equilibrated mineral oil.

Microelectrode techniques

pCO_2 microelectrode. The in situ pCO_2 of tubule fluid at the base of the papillary CD (earliest accessible portion), and tip of the collecting duct (opening of duct) (mean length of tubule = 2.2±0.4 mm) were obtained by direct puncture with a pCO_2 microelectrode 8–14-μm tip diameter. The construction, testing, electrical characteristics, and calibration of these electrodes were exactly as described previously (9, 11) except that teflon tubing (0.013 in. outside diameter, Medwire Corp., Mount Vernon, NY) was substituted for needle stock as vent tubes. Approximately four determinations of pCO_2 at each micropuncture site were performed in each experimental condition. Electrodes having a sensitivity of <57 mV/$\log_{10}$ pCO_2 were not used.

In situ pH (pH_{is}). The pH_{is} at the base and tip of the papillary CD was determined with single-barreled glass membrane pH microelectrodes of 9–12-μm tip diameter as reported previously (12). Single-barreled electrodes were employed since previous studies in our laboratory have demonstrated that the small, variable transepithelial potential difference in this segment does not adversely affect the accuracy of the pH reading obtained in this manner (12). Electrodes having a slope of <57 mV/pH U were not used. Calibration before and after in vivo use was as described previously (12).

Equilibrium pH (pH_{Eq}). Equilibrium pH, defined as the pH achieved when tubule fluid is removed from the environment of the tubule in vivo and allowed to achieve chemical equilibrium, was measured with a composite probe as described in detail previously (12). The advantages of this type of anaerobic electrode are that tubule fluid can be aspirated from the tubule by direct puncture in vivo, circumventing the necessity

for transfer of fluid to an equilibrium chamber in vitro, and assures that the tubule fluid achieves chemical equilibrium at the prevailing in vivo pCO_2. The extensive testing, manufacture, and calibration of this electrode has been described previously (12). Only electrodes having a sensitivity of at least 57 mV/pH U were employed.

Disequilibrium pH. The difference in pH_{is} and pH_{Eq} represents the disequilibrium pH as defined previously and discussed in detail recently (13). By convention, a negative pH_{Dq} (acid pH_{Dq}) denotes proton secretion, since the more acid pH_{is} occurs as a result of accumulation of H_2CO_3 in excess of the concentration expected at equilibrium. Since carbonic anhydrase is not available in the lumen or cytoplasm of cells of the papillary CD (14), H^+ secretion into bicarbonate-containing tubule fluid produces H_2CO_3 which dehydrates at the relatively slow, uncatalyzed rate. The CO_2 gas formed within the terminal nephron and collecting system is trapped because of surface-volume relationships unfavorable for diffusion. Therefore, in this as in previous studies (9, 13), the pH_{Dq} in combination with the magnitude of the increase in collecting duct CO_2 tension during bicarbonate loading were used to investigate the reliability of the U-B pCO_2 gradient as a qualitative index of collecting tubule proton secretion.

Physiologic conditions

Group I A (bicarbonate-loaded controls) (n = 15). After preparation for papillary micropuncture, replacement of surgical volume losses, and infusion with Ringer's bicarbonate as described above for 1.5 h, rats in this group were infused with 300 mM $NaHCO_3$ and 25 mM KCl at 1.8% BW/h for 2.0 h. Previous studies have demonstrated that rats infused in this manner excrete a urine pH > 7.8 U, which remains stable for several hours (9). Micropuncture was initiated after assuring stability of blood and urine pH, pCO_2, and $[HCO_3^-]$ as described previously (9).

Group I B (acid-challenge controls) (n = 12). A separate group of controls were evaluated during Ringer's bicarbonate infusion (hydropenia) for acid-base status and urine pH. After 2 30-min collection periods, 0.1 N HCl was infused at 1.5% BW for 2.0 h. After two timed urine collections and blood pH and $PaCO_2$ determinations, 3% Na_2SO_4 was infused for 1.0 h and continued during two final 30-min collection periods.

Group II A (amiloride, bicarbonate load) (n = 11). Amiloride HCl (Merck, Sharp, and Dohme, West Point, PA) was administered intraperitoneally in Ringer's bicarbonate at 5 mg/kg BW per day for 4 d. These rats were prepared for micropuncture on the fifth day as in group I, except that bicarbonate loading was accomplished with 300 mM $NaHCO_3$ (no KCl).

Group II B (amiloride, acid challenge) (n = 7). A separate group of rats received amiloride at 5 mg/kg BW for 4 d as above and urinary acidification parameters were assessed by observing urine pH during continuation of Ringer's bicarbonate infusion (hydropenia), and during acute metabolic acidosis induced by infusion of 0.1 N HCl at 1.5% BW for 2.0 h. After two timed 30-min urine collections, 0.1 N HCl was continued and in addition, 3% Na_2SO_4 was infused for 1.0 h and continued during two final 30-min collection periods.

Group III A (lithium chloride, bicarbonate load) (n = 11). Lithium chloride was prepared as a 1.6-M solution and administered intraperitoneally at 4 meq/kg per d for 4 d. Rats in this group were prepared for micropuncture as in groups I and II and were infused with 300 mM $NaHCO_3$–25 mM KCl at 1.8% BW/h for 2.0 h before initiation of micropuncture.

Group III B (lithium chloride, acid challenge) (n = 12). An additional group of rats was assessed after 4 d of lithium chloride during metabolic acidosis and after Na_2SO_4 infusion as in groups I and II.

Group IV A (post-obstructed kidney, bicarbonate load) (n = 7). Rats in this group underwent ligation of the lower portion of the left ureter 18.0 h before micropuncture after sodium methohexital anesthesia, intraperitoneally (0.1 ml/100 g BW) (Eli Lilly & Co., Indianapolis, IN). The ureter was approached by a small abdominal incision. After

Table I. Urinary Acidification Parameters in Models of DRTA

	Hydropenia			0.1 N HCL i.v.		3% Na$_2$SO$_4$ i.v.	
	pH_a	$[HCO_3^-]_a$ meq/liter	pH_u	pH_a	pH_u	pH_a	pH_u
Control (12)	7.40±0.02	24.8±0.3	5.75±0.03	7.30±0.04	5.10±0.04	7.30±0.05	5.05±0.06
Amiloride (7)	7.29±0.02*	19.7±0.5*	7.23±0.16*	7.24±0.03*	6.63±0.06*	7.23±0.01*	6.15±0.08*
LiCl (12)	7.36±0.02	21.9±0.6*	6.27±0.14*	7.28±0.02	5.78±0.05*	7.31±0.01	5.21±0.06
Post-obstructed kidney (6)	7.35±0.01	24.7±0.7	7.18±0.09*	7.30±0.01	7.11±0.07*	7.29±0.02	6.59±0.06*
Amphotericin-B (10)	7.31±0.01*	21.8±0.6*	5.98±0.08*	7.29±0.02	5.67±0.06*	7.31±0.02	5.13±0.06

Data expressed as mean values±SEM. Numbers in parentheses equal number of rats. pH_a, arterial blood pH; pH_u, urine pH. * $P < 0.001$ vs. control.

displacement of the urinary bladder, the left ureter was ligated with 1-0 silk suture and the abdominal wall and skin closed with 4-0 silk suture. After initial preparation on the next day as in the preceding groups, micropuncture was performed within 2.0 h after excising the left renal pelvis. Therefore, rats in this group were preloaded for 1.0 h with 300 mM NaHCO$_3$ plus 25 mM KCl before release of obstruction, to insure a total of 2.0 h of bicarbonate loading as in groups I and III.

Group IV B (post-obstructed kidney, acid challenge) (n = 6). An additional group of rats was assessed by the HCl-Na$_2$SO$_4$ acidification tests described for groups I, II, and III. Urine in this group was collected from the ureter of the left post-obstructed kidney and via the urinary bladder (right nonobstructed kidney) with PE-50 tubing into preweighed vials under water-equilibrated mineral oil.

Group V A (amphotericin-B, bicarbonate load) (n = 7). Amphotericin-B was injected intraperitoneally at 5 mg/kg BW/d for 16-20 d. This period of preparation was found to be necessary by preliminary studies in order to produce consistently an acidification defect while preventing renal failure (frequently present after 20 d in preliminary studies). Rats in this group were prepared for micropuncture as in the preceding group and infused with 300 mM NaHCO$_3$-25 mM KCl.

Group V B (amphotericin-B, acid challenge) (n = 10). A separate group of rats was assessed for urinary acidification after 16-20 d of amphotericin-B as described above. Arterial blood and urine pH and pCO$_2$ were determined on a blood gas analyzer (model 165, Corning Medical, Medfield, MA). Blood HCO$_3^-$ was calculated by the blood gas analyzer but urine [HCO$_3^-$] was calculated from the Henderson-Hasselbalch equation ($\alpha = 0.0309$ and apparent pK corrected for ionic strength as described previously) (9).

The results are expressed as mean values±SEM in each group.

Statistical significance was calculated using the t test for paired or unpaired data as appropriate.

Results

Systemic acid-base and whole kidney values. The acid-base status and urine pH in each group before bicarbonate loading is displayed in Table I. A mild metabolic acidosis was observed in groups II and V (E vs. C, $P < 0.001$) but was more severe in group II rats (amiloride). The explanation for the mild acidemia in the amphotericin-B group can be attributed to the combined effect of mild hypobicarbonatemia and an inappropriate ventilatory response. All groups had evidence of a urinary acidification defect since the initial urine pH was inappropriately alkaline (Table I). Moreover, after infusion of 0.1 N HCl at 1.5% BW/h for 2 h, urine pH did not decrease to <5.5 in groups II-V ($P < 0.001$). Finally, after infusion of 3% Na$_2$SO$_4$, urine pH decreased below 5.5 in groups III (LiCl) and V (amphotericin-B) but not after amiloride (group II) or ureteral obstruction (group IV).

The systemic acid-base and right whole kidney values observed during bicarbonate-loading are displayed in Table II. As expected, rats in each group developed metabolic alkalosis in response to bicarbonate loading. The degree of metabolic alkalosis was similar in all groups (I-V) ($P > 0.05$). Furthermore, the urine pH was near 8.0 in all groups. Despite the significantly lower urine pH in group II (7.81 vs. 7.97, $P < 0.001$), all rats

Table II. Systemic Acid-Base and Right Whole Kidney Data—Bicarbonate Loading

Experimental group	Blood			Urine			
	pH_a	$[HCO_3^-]$ meq/liter	pCO_2 mmHg	pH_u	pCO_2 mmHg	$[HCO_3^-]$ meq/liter	U-B pCO_2 mmHg
Control (15)	7.50±0.01	33.6±1.5	45±2	7.97±0.02	118±4	211.3±4.1	68.4±4.2
Amiloride (11)	7.53±0.02	31.6±1.2	39±1*	7.81±0.04*	69±4*	115.6±11.4*	31.0±3.5*
Lithium chloride (11)	7.52±0.02	32.6±1.0	42±2	8.02±0.03	85±5*	195.8±6.3	40.0±5.0*
Post-obstructed kidney (7)	7.52±0.03	35.4±1.9	45±2	7.99±0.02	90±4*	208.5±10.3	41.0±6.0*
Amphotericin-B (7)	7.52±0.04	36.2±1.8	45±2	8.01±0.50	103±7	218.3±3.8	58.0±6.3

Data expressed as mean values±SEM. Numbers in parentheses equal number of rats. * $P < 0.001$ vs. control.

Table III. Micropuncture Data: Disequilibrium pH

Experimental group	Base CD			Tip CD		
	pH_{is}	pH_{Eq}	pH_{Dq}	pH_{is}	pH_{Eq}	pH_{Dq}
Control	7.76±0.04	7.99±0.04	−0.24±0.04* (31)	7.74±0.04	7.99±0.02	−0.26±0.05* (32)
Amiloride	7.79±0.07	7.74±0.07	+0.01±0.03 (21)	7.61±0.06	7.65±0.05	−0.08±0.03 (18)
Lithium chloride	7.75±0.04	7.77±0.05	+0.03±0.05 (20)	7.81±0.04	7.80±0.04	−0.04±0.02 (21)
Post-obstructed						
kidney	7.77±0.03	7.72±0.03	+0.08±0.03 (25)	7.73±0.03	7.68±0.03	+0.02±0.06 (25)
Amphotericin-B	7.80±0.07	7.95±0.05	−0.16±0.05‡ (18)	7.71±0.06	7.78±0.04	−0.11±0.05§ (22)

Data expressed as mean values±SEM. Numbers in parentheses equal number of determinations. * $P < 0.001$ vs. 0.0; ‡ $P < 0.01$ vs. 0.0; § $P < 0.05$.

excreted a highly alkaline urine, so that nonbicarbonate buffer would not be expected to contribute to the increase in urine pCO_2 above systemic arterial blood levels (2, 7, 9). Urine pCO_2 was significantly higher than systemic arterial levels in all experimental groups (U-B pCO_2 vs. 0, $P < 0.001$). Nevertheless, the urine pCO_2, and U-B pCO_2 was significantly less than corresponding values in controls in groups II, III, and IV ($P < 0.001$). The U-B pCO_2 after amphotericin-B (group V), however, was not different than in control rats ($P > 0.05$).

Micropuncture data. The micropuncture data are displayed in Tables III and IV and Figs. 1 and 2. The pH data displayed in Table III will be considered first. Findings for pH_{is}, pH_{Eq}, and pH_{Dq} ($pH_{Dq} = pH_{is} - pH_{Eq}$) are displayed for both the base and tip collecting duct (BCD and TCD) micropuncture sites. Values for disequilibrium pH significantly different from 0.0 U are indicated by an asterisk. In control rats during bicarbonate loading a significant acid pH_{Dq} was observed at both the base and tip ($P < 0.001$) (Fig. 1). After amiloride, lithium chloride, and ureteral obstruction, however, the pH_{Dq} was obliterated as evidenced by values for in situ pH and equilibrium pH which were indistinguishable. Therefore, despite high delivery of bicarbonate to this segment, proton secretion was not of sufficient magnitude in these groups to result in the formation of H_2CO_3 in excess of the concentration predicted at chemical equilibrium. Conversely, however, amphotericin-B was associated with a significant acid pH_{Dq} (compared with

Table IV. Micropuncture Data: pCO_2

	Base CD	Tip CD
	mmHg	mmHg
Experimental group		
Control	73.2±3.7 (25)	116.9±3.2 (29)
Amiloride	46.8±2.7* (44)	66.9±2.5* (42)
Lithium chloride	68.8±3.1 (40)	90.3±3.0* (36)
Post-obstructed		
kidney	54.9±4.2* (26)	77.6±3.6* (23)
Amphotericin-B	110.4±6.8* (28)	119.8±6.4 (28)

Numbers in parentheses equal number of determinations.
* $P < 0.001$ vs. control.

0.0 pH U) at both the base (−0.16±0.05, $P < 0.01$) and tip of the CD (−0.11±0.05, $P < 0.05$).

The pCO_2 data for both the BCD and TCD are displayed in Table IV and Fig. 2. Note first that in each group the pCO_2 at the tip of the papillary CD obtained by micropuncture with the pCO_2 microelectrode was similar to the pCO_2 in final urine from the right nonexperimental kidney measured by a conventional macroelectrode (Table II). As noted in previous studies (9), the pCO_2 increased significantly from BCD to TCD in control rats (group I) ($P < 0.001$). In group II (amiloride), the pCO_2 at both base (46.8±2.7 mmHg) and tip CD (66.9±2.5 mmHg) was significantly lower than in controls at both sites ($P < 0.001$). Nevertheless, pCO_2 increased significantly from BCD to TCD. In group III rats (LiCl), there was a significantly lower pCO_2 at the TCD than in controls (90.3±3.0 vs. 116.9±3.2 mmHg) ($P < 0.001$). However, the pCO_2 at the BCD (68.8±3.1 mmHg) did not differ from controls (73.2±3.7 mmHg) ($P > 0.05$). After ureteral obstruction, however, pCO_2 was significantly lower at both the BCD (54.9±4.2) and TCD (77.6±3.6 mmHg) ($P < 0.001$), respectively. In contrast to the other experimental models of DRTA, amphotericin-B was associated with a papillary pCO_2 which was indistinguishable from the pCO_2 observed in controls ($P > 0.05$). Indeed, the pCO_2 observed at the BCD was significantly greater than that observed in control rats ($P < 0.001$), so that the pCO_2 profile from base to tip was no longer significant (110.4±6.8–119.8±6.4 mmHg, $P > 0.05$). Since the mean urine bicarbonate concentration was lower in the chronic amiloride rats (group II) than in the other groups (Table II), we examined three additional rats in which the mean urine bicarbonate concentration was 197.5±10.7 meq/liter. The disequilibrium pH of the BCD and TCD was +0.02±0.02 and −0.03±0.02, respectively. Further-
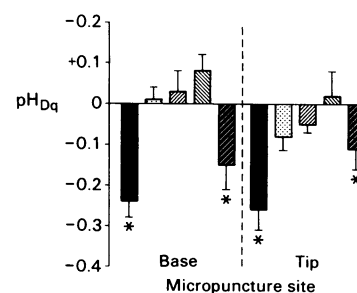
Figure 1. Disequilibrium pH at the base and tip of the papillary CD. Bars arranged as follows: controls, amiloride, LiCl, POK, and amphotericin-B. Asterisk denotes $P < 0.05$ vs. 0.0 pH_{Dq}.
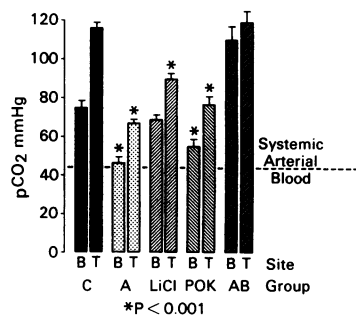
Figure 2. pCO_2 determined by microelectrode at base (B) and tip (T) collecting duct sites in each group (control, amiloride, lithium chloride, post-obstructed kidney, and amphotericin-B. Asterisk indicates values significantly less ($P < 0.001$) than control corresponding value.

more, the pCO_2 was 53.9 ± 3.0 at the BCD and 68.5 ± 3.0 at the TCD. Neither value differed from the micropuncture findings reported in the rats with lower urine bicarbonate concentration.

Discussion

Numerous provocative tests have been employed in man and experimental animals to investigate urinary acidification. The urine-to-blood pCO_2 difference has been applied widely as a qualitative index of distal nephron proton secretion (2, 4, 6, 15–18). Previous studies in our laboratory have demonstrated that the increase in urine pCO_2 observed in highly alkaline urine was associated with an acid pH_{Dq} which was reduced by carbonic anhydrase infusion (9). Although these findings support the view that the U-B pCO_2 can be employed as a qualitative index of distal nephron proton secretion, a similar direct evaluation in models of deranged distal nephron acidification have not been available previously.

The present investigation was designed to measure pH_{Dq} and pCO_2 in the collecting duct in several models of DRTA in the rat to determine if a reduction in U-B pCO_2 is synonymous with a reduction in proton secretion. Several new findings have emerged from these studies. First, amiloride, lithium chloride, and the post-obstructed kidney, all employed commonly as models of defective distal nephron acidification, were associated uniformly with an obliteration of the acid pH_{Dq} and a reduction in pCO_2 in the papillary CD. Second, amphotericin-B-treated rats with evidence of an acidification defect of the gradient type continued to maintain an acid pH_{Dq} as well as the ability to generate an elevated pCO_2 in the papillary CD. Therefore, these findings support utilization of the U-B pCO_2 gradient to document impaired net proton secretion in the terminal nephron in voltage dependent and/ or secretory forms of distal renal tubular acidosis (RTA) and support the view that proton secretion in the gradient form of RTA is not impaired.

Several types of DRTA, secretory, voltage dependent, and gradient, have been classified by both in vivo clearance techniques and by direct exposure of acidifying epithelia in vitro to drugs known to compromise acidification (2, 19). From such in vivo-in vitro comparisons, there has not been uniformity regarding the type of mechanism proposed to explain the defect observed in experimental models. For example, in this and previous studies, chronic lithium chloride administration in rats resulted in the development of a hyperchloremic, normokalemic metabolic acidosis, an inappropriately alkaline urine pH, and a low U-B pCO_2 (20, 21). The inability to lower urine pH appropriately with spontaneous acidosis or after an

acid challenge (below 5.5) is corrected by infusion of sodium sulfate in this model (21). Based on this constellation of findings, the defect induced by lithium was initially assumed to be due to a gradient defect, i.e., an excessive back-diffusion of acid as had been proposed for amphotericin-B (21). However, it was emphasized by Roscoe and associates (20) that the failure to observe an appropriate increase in U-B pCO_2 after lithium was best explained by a decrease in distal nephron proton secretion (20). Finally, studies in the turtle urinary bladder under open-circuited conditions revealed that lithium impaired proton secretion by virtue of a detrimental effect on the electrical gradient favoring H^+ secretion (22). Thus, it was concluded that lithium impaired urinary acidification, not as a consequence of backleak of acid but by mediating a voltage dependent impairment in proton secretion (22). It was also suggested that the normal response to sodium sulfate in the lithium model could be explained as a result of enhanced distal delivery of sodium restoring lumen-negative potential difference (2, 19, 22). The findings in the present study during chronic lithium administration are compatible with the view that lithium impairs net acid secretion. Our finding that the acid pH_{Dq} is indistinguishable from zero and is associated with a reduction in pCO_2 in the papillary CD demonstrates, for the first time, that net proton secretion is impaired in a nephron segment before the first accessible portion of the papillary CD. The segment of the nephron in which lithium impairs proton secretion and the mechanism by which this occurs in vivo has not been established unequivocally. Recently, however, it has been reported that high concentrations of lithium chloride (40 mM) decreased transepithelial voltage (-11.6–0.4 mV) and bicarbonate reabsorption (10.8–4.2 pmol mm^{-1} min^{-1}) in rabbit cortical collecting tubules perfused in vitro (23). No effect on transepithelial voltage or bicarbonate reabsorption was observed in the medullary CD (23). It was suggested that H^+ secretion in the cortical (but not the medullary) CD was dependent, in part, on the negative transepithelial potential generated by outward active Na^+ transport, and that this process was impaired by lithium (23). This hypothesis is also compatible with results obtained during application of LiCl to acidifying turtle bladder in vitro (22). The findings in our study, therefore, are the first to demonstrate directly a relationship between the reduction in pCO_2 and disequilibrium pH in an in vivo model of voltage-dependent DRTA.

Similarly, chronic amiloride administration, another widely accepted experimental model of voltage-dependent DRTA, was associated with a reduction in papillary pCO_2 and an obliteration of pH_{Dq}. Amiloride has been demonstrated to have an effect on H^+ secretion similar to lithium in the turtle urinary bladder in the open-circuited state, but appears to have no direct effect on H^+ secretion independent of voltage (24, 25). The effect of amiloride on acidification in vivo assessed by clearance and balance techniques has been reported in the dog (26) and rat (25). Both studies demonstrated that chronic amiloride administration was associated with the development of impaired distal acidification, an inappropriately high urine pH that did not respond to sodium sulfate infusion, and an abnormally low urine-to-blood pCO_2. The amiloride model therefore differs from the lithium model in several respects. Interestingly, despite an increase in Na^+ delivery to the distal nephron (as Na_2SO_4), the voltage-dependent defect was not corrected (25). Furthermore, this sustained impairment in distal nephron proton secretion has been demonstrated to

be independent of alterations in potassium balance (26). Although modest hyperkalemia existed in chronic amiloride-treated rats in our study as well (K^+ = 5.2±0.4 meq/liter), it seems highly unlikely that this would have an effect on the pH_{Dq} or pCO_2, since hyperkalemia alters net acid excretion primarily by influencing the production of ammonia. Stone and associates (27) demonstrated that amiloride (5×10^{-5} M) had no effect on rabbit medullary collecting tubule bicarbonate transport or transepithelial voltage in vitro in the presence or absence of aldosterone (10^{-6} M). There are no studies available examining the effect of amiloride on bicarbonate reabsorption in inner medullary or papillary CD in vitro or in vivo. However, Ullrich and Papavassiliou (28) have demonstrated that amiloride (10^{-4} M) markedly reduces volume flux in the papillary CD and that the majority of Na^+ transport in this segment occurs via an amiloride-sensitive Na^+ channel. Therefore, the possibility that amiloride exerts an effect in this segment on acidification, pH_{Dq} and pCO_2 must be considered as a possibility in our study. The precise segment of the nephron responsible for this combination of findings remains speculative, however, because of inaccessibility to micropuncture of all nephron segments involved.

The classification of the defect associated with the post-obstructed kidney is more problematic, however. Previous whole kidney studies have revealed that the U-B pCO_2 gradient is low during bicarbonate loading, the urine pH inappropriately high during systemic acidosis, and that there is no response to sodium sulfate (29–31). Such findings are compatible with the clearance data in the present study (Table I). In addition, in response to phosphate administration, the U-B pCO_2 failed to increase, while urinary potassium excretion was abnormally low even during sodium sulfate administration (31). These findings and the results of micropuncture studies in vivo (29) have led to the classification of the acidification defect in the post-obstructed kidney as a secretory or pump defect (2). However, a more recent review (19) noted the similarities of the results obtained with amiloride and the post-obstructed kidney and suggested that the latter is an example of a voltage-dependent defect. Unfortunately, the parameters measured in the present study do not allow the distinction between these two types of disorders, since the effect on pH_{Dq} and pCO_2 was identical to the results with amiloride and lithium. However, perfusion studies in vitro of tubules harvested from previously obstructed kidneys have demonstrated that the transepithelial potential difference in the cortical collecting tubule decreases from −10±2 mV to +3±4 mV, and that volume reabsorption in the presence of antidiuretic hormone is markedly impaired (32). These findings appear to support the general formulation of a voltage-dependent defect after obstruction. Bicarbonate transport was not evaluated in the in vitro study, however, and the medullary collecting tubule has not been examined similarly. A previous micropuncture study (29) in which this experimental model was employed supports a contribution to the acidification defect by the portion of the nephron between late distal tubule and final urine as well as the distal tubule per se. Therefore, taken together, our findings and other studies available in which the post-obstructed kidney was evaluated, support a defect in acidification due either to impaired proton secretion per se or to a secondary compromise of proton secretion due to a voltage-dependent defect. Nevertheless, of the tests available which can be applied in whole kidney studies, our findings strongly support the application of the U-B pCO_2 gradient during excretion of an alkaline urine as a reliable and sensitive index of impaired distal nephron proton secretion in both voltage dependent and/or secretory defects, as exemplified by the findings with lithium, amiloride, and ureteral obstruction.

In sharp contrast to the results obtained in these two experimental models, the effect of chronic amphotericin-B on pH_{Dq} and pCO_2 in the papillary CD did not differ from controls with intact acidification. In the present study, a defect in acidification was demonstrated in this group by the inability to lower urine pH during acute acidosis, and the appropriate response to sodium sulfate (i.e., urine pH fell to <5.5) (Table I). During bicarbonate loading, however, the U-B pCO_2 gradient increased as in controls with intact urinary acidification (Table II). This finding is compatible with findings reported previously by others (33, 34). As in the latter study, the rats in the present investigation received amphotericin-B for a prolonged period (16–20 d), but did not have evidence of significant renal failure (blood urea nitrogen = 16±3, creatinine = 0.6±0.02 mg/dl). Based on similar observations from whole kidney studies in the amphotericin or gradient model, Roscoe and associates (18) suggested that this finding (normal U-B pCO_2) supported the validity of the U-B pCO_2 as an index of intact proton secretion in the amphotericin defect. Others have suggested, in contrast, that a permeability defect such as that produced by amphotericin could be associated with increased back-diffusion of carbonic acid and thus a low U-B pCO_2 (2, 19). The rationale for the classification of amphotericin-B as a gradient defect is based on observations reported by Steinmetz and associates (35). In the turtle bladder, in vitro amphotericin-B was shown to impair the ability to maintain a pH gradient between serosa and mucosa (35). In a gradient defect, such as that induced by amphotericin, there is no a priori reason to expect a reduction in U-B pCO_2 based on these in vitro observations, however. Indeed, our findings may provide an explanation for the observation of a normal U-B pCO_2 in this model.

In the present study, the acid pH_{Dq} was equal to −0.16±0.06 at the base CD and remained significantly greater than zero along the length of the papillary CD. This may be interpreted as evidence for continued proton secretion in the terminal nephron. In the presence of high bicarbonate delivery to the papillary CD, secreted hydrogen ions may be "trapped" by HCO_3^- and form H_2CO_3 which is dehydrated at the uncatalyzed rate to produce CO_2. Thus, the urine pCO_2 would not be reduced if high bicarbonate delivery prevents H_2CO_3 back-diffusion. Alternatively, HCO_3^- could be secreted from blood to lumen and not only produce a negative pH_{Dq} but explain, as well, the increase in pCO_2 in final urine and papillary CD. It has been documented clearly that bicarbonate may be secreted by the cortical, but not the medullary collecting tubule, in rabbit and rat tubules perfused in vitro (36–38). The magnitude of bicarbonate secretion has been observed to increase after bicarbonate loading in vivo (38). Unfortunately, we are unable to determine with papillary micropuncture techniques if bicarbonate secretion could be occurring in this model beyond the late distal tubule before the base papillary collecting tubule. Since the concentration of bicarbonate in the collecting duct in the present study is ~200 mM, the concentration gradient required for passive secretion seems unlikely. The effect of amphotericin-B on cortical or medullary collecting tubule bicarbonate transport has not been examined

in vitro. Nevertheless, it is clear from the present study that the U-B pCO_2 technique as applied in clearance studies indicates intact proton secretion in models of gradient or back-leak distal RTA. Therefore, an inappropriately alkaline urine pH after an acid challenge which is corrected by Na_2SO_4 infusion (<5.5 U) must be coupled with the demonstration of a normal U-B pCO_2 to reliably characterize this type of DRTA. Interestingly, in this regard, the observation by Halperin and colleagues (6) that the U-B pCO_2 is often decreased in classical distal RTA in man suggests that this disorder is not due to a gradient defect, as first proposed, but a result of a voltage or secretory defect. Nevertheless, we consider this view somewhat speculative until a suitable experimental model of classical distal RTA can be developed and examined in more detail.

In summary, we have demonstrated that amiloride, lithium, and the post-obstructed kidney impair urinary acidification and net H^+ secretion in the terminal nephron. The U-B pCO_2 gradient, as applied in whole kidney studies, appears to demonstrate the defect in distal nephron proton secretion reliably. Amphotericin-B, however, impairs the ability to maintain a pH gradient in the collecting tubule, but the ability to increase urine pCO_2 during bicarbonate loading remains intact. While the explanation for this latter observation is not entirely clear, the findings suggest that the U-B pCO_2 gradient must be combined with other dynamic tests of urinary acidification in order to characterize reliably the acidification defect in the gradient form of DRTA.

Acknowledgments

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