The effects of respiratory alkalosis and acidosis on net bicarbonate flux along the rat loop of Henle in vivo

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Unwin, R., R. Stidwell, S. Taylor, and G. Capasso. The effects of respiratory alkalosis and acidosis on net bicarbonate flux along the rat loop of Henle in vivo. *Am. J. Physiol.* 273 (*Renal Physiol.* 42): F698–F705, 1997.—We have studied the effects of acute respiratory alkalosis (ARALK, hyperventilation) and acidosis (ARA, 8% CO_2), chronic respiratory acidosis (CRA; 10% CO_2 for 7–10 days), and subsequent recovery from CRA breathing air on loop of Henle (LOH) net bicarbonate flux (J_{HCO_3}) by in vivo tubule microperfusion in anesthetized rats. In ARALK blood, pH increased to 7.6, and blood bicarbonate concentration ($[HCO_3^-]$) decreased from 29 to 22 mM. Fractional urinary bicarbonate excretion (FE_{HCO_3}) increased threefold, but LOH J_{HCO_3} was unchanged. In ARA, blood pH fell to 7.2, and blood $[HCO_3^-]$ rose from 28 to 34 mM; FE_{HCO_3} was reduced to <0.1%, but LOH J_{HCO_3} was unaltered. In CRA, blood pH fell to 7.2, and blood $[HCO_3^-]$ increased to >50 mM, whereas FE_{HCO_3} decreased to <0.1%. J_{HCO_3} was reduced by ~30%. Bicarbonaturia occurred when CRA rats breathed air, yet LOH J_{HCO_3} increased (by 30%) to normal. These results suggest that LOH J_{HCO_3} is affected by the blood-to-tubule lumen $[HCO_3^-]$ gradient and HCO_3^- backflux. When the usual perfusing solution at 20 nl/min was made HCO_3^- free, mean J_{HCO_3} was -34.5 ± 4.4 pmol/min compared with 210 ± 28.1 pmol/min plus HCO_3^-. When a low-NaCl perfusate (to minimize net fluid absorption) containing mannitol and acetazolamide (2×10^{-4} M, to abolish H^+-dependent J_{HCO_3}) was used, J_{HCO_3} was -112.8 ± 5.6 pmol/min. Comparable values for J_{HCO_3} at 10 nl/min were -35.9 ± 5.8 and -72.5 ± 8.8 pmol/min, respectively. These data indicate significant backflux of HCO_3^- along the LOH, which depends on the blood-to-lumen $[HCO_3^-]$ gradient; in addition to any underlying changes in active acid-base transport mechanisms, HCO_3^- permeability and backflux are important determinants of LOH J_{HCO_3} in vivo.

loop of Henle; bicarbonate transport; respiratory acidosis; respiratory alkalosis; permeability; backflux

THE KIDNEY PLAYS an important role in the homeostatic response to acute and chronic acid-base disturbances by changes in reabsorption of filtered bicarbonate in exchange for secreted H^+ and in the excretion of nonbicarbonate buffers such as phosphate and ammonium. Several groups have shown that the proximal tubule is the major site of bicarbonate reabsorption (2, 3, 30) and that net transport adjusts to systemic acid-base changes (13, 14, 28, 31). However, more distal nephron segments may also participate in the renal adaptation to acid-base disturbances (26). Among the distal nephron segments, the loop of Henle (LOH) reabsorbs ~10–15% of the filtered bicarbonate (6, 15). We have shown previously that bicarbonate transport in this segment in vivo is not saturated under normal acid-base conditions and is mediated by luminal Na^+/H^+ exchange and H^+-adenosinetriphosphatase (H^+-ATPase) (8).

The LOH is composed of several subsegments, including the thick ascending limb (TAL). Good (21) has shown that the perfused TAL in vitro reabsorbs bicarbonate and is responsible for a significant portion of total LOH bicarbonate transport. He has also reported that bicarbonate reabsorption in the TAL is stimulated by metabolic acidosis and inhibited by metabolic alkalosis (22). We have demonstrated similar effects of metabolic acid-base disturbances along the LOH in vivo (9). However, the effect of respiratory acid-base changes on the LOH has not been investigated. In contrast to metabolic acid-base disturbances, in respiratory abnormalities, the blood bicarbonate concentration decreases in alkalosis and increases in acidosis. In low-resistance leaky epithelia, like the proximal tubule and part of the LOH, the blood-to-tubule lumen bicarbonate concentration gradient is probably also an important determinant of net bicarbonate absorption in vivo.

To investigate the response of the LOH under conditions of altered respiratory acid-base conditions and fixed-end proximal delivery of bicarbonate, we have done a tubule microperfusion study on the effect of acute and chronic respiratory acid-base manipulations on bicarbonate reabsorption along the rat LOH in vivo. Our data show that acute respiratory alkalosis and acidosis have no effect on LOH bicarbonate transport, whereas, in chronic respiratory acidosis, bicarbonate absorption is depressed but rises during partial recovery when blood bicarbonate concentration falls as urinary bicarbonate excretion increases. The microperfusion data can be explained in terms of changes in bicarbonate backflux (from blood to tubule fluid) as a consequence of alterations in the blood-to-lumen bicarbonate concentration gradient. The results of additional microperfusion experiments designed to minimize the active transport component of bicarbonate absorption and to assess the magnitude of bicarbonate backflux along the LOH are consistent with this conclusion.

METHODS

Preparation of animals. We did experiments on a total of 45 male Sprague-Dawley rats (220 ± 4 g body wt) kept in cages at 21°C in controlled daylight and fed until the day of the study. They were anesthetized intraperitoneally with Inactin (Promonta), using a dose of 120 mg/kg body wt, tracheostomized, placed in the right lateral position on a thermoregulated table (37°C), and prepared for micropuncture in the usual way. In brief, the right carotid artery was catheterized to record blood pressure and take blood for measurements of

hematocrit, arterial pH, and blood gases. The left jugular vein was cannulated with PE-50 tubing and used for intravenous infusion via a syringe pump (Razel, Semat Technical) of a Ringer-saline solution (125 mM NaCl + 25 mM $NaHCO_3$) at 4 ml/h. The left kidney was exposed through a flank incision, made free of perirenal fat, and immobilized in a Lucite chamber with 3% agar in 0.9% saline. The kidney was bathed with prewarmed (37°C) paraffin oil. The bladder and left ureter were catheterized with PE-90 and PE-10 tubing, respectively, for collection of urine.

Tubule microperfusion. We performed continuous microperfusion of superficial LOHs in vivo to measure bicarbonate and fluid transport under conditions of fixed flow rate and bicarbonate delivery. Briefly, a perfusion pipette was inserted into the last surface loop of proximal tubule, and a castor oil block was placed upstream of the perfusion pipette. Microperfusion was started at 20 nl/min with a thermally shielded microperfusion pump (Hampel, Frankfurt, Germany). The perfusion solution contained (in mM) 128 NaCl, 13 $NaHCO_3$, 3.8 KCl, 1 $MgCl_2$, 0.38 NaH_2PO_4, and 1.62 Na_2HPO_4 (280 mosmol/kgH_2O). FD & C blue dye (0.07%) and 12.5 μCi/ml [^{14}C]inulin were added to the perfusion solution. Net fluid flux (J_v) and net bicarbonate transport rate (J_{HCO_3}) were measured under the following conditions: *1*) acute respiratory alkalosis induced by mechanical hyperventilation [SAR 830 mechanical ventilator, CWE; tidal volume (V_T) = 10.5 ml, respiratory rate = 75–80 breaths/min], *2*) acute respiratory acidosis induced by mechanical ventilation with 8% CO_2 (V_T = 7.5, respiratory rate = 40 breaths/min), and *3*) chronic respiratory acidosis (CRA) produced by maintaining animals in a 10% CO_2 atmosphere for 7–10 days and subsequently during recovery when breathing air. To maintain the acidotic state during micropuncture, the rats continued to breath 10% CO_2 through a purpose-designed, positive-pressure hood delivering a constant 10% CO_2-90% air mixture. This hood was removed during the "recovery" phase. In acute respiratory acidosis, to assist mechanical ventilation and to maintain normoxia and hemodynamic stability, it was necessary to use the neuromuscular blocking agent gallamine triethiodide (Rhone-Poulenc Rorer; 5 mg/kg iv bolus followed by 15 $mg \cdot kg^{-1} \cdot h^{-1}$ iv infusion) in addition to Inactin anaesthesia. Tubule microperfusion did not begin until at least 45 min after surgery, when the animal was equilibrated and stable and at least 30 min after the subsequent acute changes in mechanical ventilation and respiratory acid-base status.

To estimate whole-loop bicarbonate permeability, we did separate microperfusion experiments in normal rats with a modified solution previously reported by McKay and Peterson (32) to prevent net fluid and chloride reabsorption along the LOH, perfusing at 10 and 20 nl/min. This was bicarbonate free and contained (in mM) 93 NaCl, 5 KCl, 4.1 $MgSO_4$, 1 NaH_2PO_4, 1 $CaCl_2$, 4 urea, 16 sodium gluconate, 71 mannitol, 0.2 acetazolamide, FD & C blue dye (0.05%), and 12.5 μCi/ml [^{14}C]inulin (300 mosmol/kgH_2O).

Except for the CRA group, each treatment group served as its own control. Transport data are given per individual loop, since it has been shown (43) that the cortical LOH is a nephron segment of essentially constant length (~6–7 mm).

Whole kidney clearance. In the same groups of animals, glomerular filtration rate (GFR) and sodium, potassium, and bicarbonate excretion were measured in the micropunctured kidney. After a priming dose of 20 μCi [^{3}H]inulin in 0.5 ml 0.9% saline, the maintenance intravenous Ringer-bicarbonate solution delivered [^{3}H]inulin at 20 μCi/h. After a 45-min equilibration, the first of three to five 30 to 45 min urine collections began. Carotid artery blood samples were taken at the start and end of each experiment and at ~1 h after any change in acid-base status.

Analytical methods. Tubule fluid total CO_2 concentration was measured by microcalorimetry (Picapnotherm, World Precision Instruments). To avoid loss of CO_2, all mineral oil used (to bathe kidney surface, to collect tubule fluid samples, and to cover samples for measurement) was equilibrated to cortical carbon dioxide tension (P_{CO_2}) values (16) with a solution containing 100 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid buffer and 48 mM $NaHCO_3$ and equilibrated with 6.7% of CO_2. Each sample analysis was bracketed by running standards of known $NaHCO_3$ concentration. The blood acid-base status of each animal and calculated blood bicarbonate concentration were measured with a blood-gas analyzer (Corning model 178). [^{14}C]inulin radioactivity was measured by a liquid scintillation counter (Parkard Tri-Carb 2000CA). Plasma Na^+ concentration [Na^+] and K^+ concentration ([K^+]) were measured by ion-sensitive microelectrodes (Corning model 614).

Calculations and statistical analysis. Single-kidney GFR and fractional electrolyte excretion were calculated using standard clearance formulas.

In the micropuncture experiments, the perfusion rate achieved in vivo was obtained from the rate of fluid collected from the early distal tubule, multiplied by the ratio of inulin concentrations in collected and perfused fluids. The perfusion pump was calibrated by timed collections of perfusion fluid delivered directly into counting vials for measurements of [^{14}C]inulin concentrations; calculated J_v (in nl/min) was the difference between perfusion and collection rates. J_{HCO_3} (in pmol/min) was calculated from the amount of bicarbonate delivered in the perfusion pipette minus the amount collected in the collection pipette, according to the following formulas: $V_p = (In_c/In_p) \times (V_c)$, where V_p is the calculated perfusion rate, In_c and In_p are the collected and perfused inulin counts, respectively, and V_c is the collection rate; $J_v = V_p - V_c$; and $J_{HCO_3} = (C_p \times V_p) - (C_c \times V_c)$, where C_p and C_c are the bicarbonate concentrations of the perfused and collected fluids.

A sample collection was considered acceptable if V_p was within 15% of the calibrated microperfusion pump rate.

Statistical analysis was by one-way analysis of variance (ANOVA). For J_{HCO_3}, because it is load dependent, one-way analysis of covariance (ANCOVA) was used with bicarbonate load as the covariate (8). ANOVA and ANCOVA were followed by comparison with the appropriate control (post hoc if $P < 0.05$) using the least significant difference test. A value of $P < 0.05$ was considered to be statistically significant. Data are given as means ± SE.

RESULTS

Blood gases and electrolytes. Table 1 summarizes the arterial blood composition data of the animals studied in each treatment group. Values of pH, P_{CO_2}, and blood HCO_3^- concentration ([HCO_3^-]) were as expected for each respiratory acid-base disturbance. Mechanical ventilation on air was associated with a decrease in plasma [K^+]. In acute respiratory alkalosis, the rise in blood pH was associated with a fall in P_{CO_2}, blood [HCO_3^-], and plasma [K^+]. In acute respiratory acidosis, a low blood pH was associated with a high P_{CO_2} and the formation of new bicarbonate with an increased blood [HCO_3^-] but no significant change in plasma [K^+]. In CRA, blood pH was reduced, P_{CO_2} was high, and blood [HCO_3^-] was significantly elevated; plasma [K^+] was

Table 1. *Arterial blood pH, P_{CO_2}, calculated bicarbonate concentration, hematocrit, and plasma sodium and potassium concentrations during spontaneous and mechanical ventilation*

Treatment	Group	*n*	Arterial Blood pH, mmHg	Arterial Blood P_{CO_2}, mmHg	Blood $[HCO_3^-]$, mM	Hematocrit	P[Na^+], mM	P[K^+], mM	Mean Arterial BP, mmHg
				Control					
Spontaneous ventilation	*1*	9	7.436 ± 0.007	43.0 ± 0.8	28.8 ± 0.4	43 ± 1	144.1 ± 0.9	4.01 ± 0.12	124.3 ± 2.5
Mechanical ventilation, air	*2*	9	7.448 ± 0.006	41.4 ± 1.4	28.4 ± 0.8	44 ± 1	143.7 ± 0.7	3.09 ± 0.08‡	110.3 ± 3.6‡
				Acute respiratory alkalosis					
Mechanical hyperventilation, air	*3*	9	7.616 ± 0.028*	23.5 ± 3.3*	21.9 ± 0.9*	41 ± 1*	143.7 ± 0.5	3.64 ± 0.06*	128.6 ± 3.4
				Acute respiratory acidosis					
Mechanical ventilation, 8% CO_2	*4*	9	7.188 ± 0.011†	87.6 ± 1.4†	33.8 ± 0.9†	42 ± 1	146.3 ± 0.8†	3.30 ± 0.11	102.9 ± 3.6
				Chronic respiratory acidosis					
Spontaneous ventilation, 10% CO_2	*5*	8	7.188 ± 0.028‡	138.5 ± 11.6‡	51.5 ± 1.1‡	43 ± 1	147.2 ± 0.5‡	5.11 ± 0.22‡	108.5 ± 3.0‡
Spontaneous ventilation, air	*6*	8	7.457 ± 0.015§	61.0 ± 3.6§	42.0 ± 1.2§	44 ± 1	145.6 ± 0.4§	3.88 ± 0.10§	110.5 ± 2.4

Values are means ± SE; *n* = no. of animals. Ventilations were on air, acute respiratory alkalosis (mechanical hyperventilation), acute respiratory acidosis (mechanical ventilation on 8% CO_2), chronic respiratory acidosis (spontaneous breathing of 10% CO_2), and during partial recovery from CO_2 adaptation breathing air (treatment *groups 1–6*). Blood $[HCO_3^-]$, blood bicarbonate concentration; P[Na^+], plasma sodium concentration; P[K^+], plasma potassium concentration; BP, blood pressure. *$P < 0.05$ compared with *groups 3* vs. *1*. †$P < 0.05$ comparing *groups 4* vs. *2*. ‡$P < 0.05$ compared with *group 1* (control) by least significant difference (LSD) testing after first comparing *groups 1, 2,* and *5* by analysis of variance (ANOVA) ($P < 0.05$). §$P < 0.05$ comparing *groups 6* vs. *5*.

also significantly increased, but, during partial recovery (breathing air), it fell.

Renal clearance data. Table 2 shows the effect of each respiratory acid-base manipulation on GFR and urinary excretion of Na^+, K^+, and HCO_3^-. In the same group of rats, mechanical ventilation per se had a small effect on GFR, reducing it from 0.72 ± 0.10 (data not shown in Table 2) to 0.56 ± 0.07 ml·min^{-1}·100 g^{-1} ($P >$

Table 2. *Urine flow rate, GFR, and fractional excretions (FEN_a, FEK, and FE_{HCO_3}) of micropunctured kidney during spontaneous and mechanical ventilation*

Treatment	Group	*n*	Urine Flow Rate, µl·min^{-1}·100 g^{-1}	GFR, ml·min^{-1}·100 g^{-1}	$U_{Na}V$, µmol·min^{-1}·100 g^{-1}	U_KV, µmol·min^{-1}·100 g^{-1}	$U_{HCO_3}V$, µmol·min^{-1}·100 g^{-1}	FE_{Na}, %	FE_K, %	FE_{HCO_3}, %
					Control					
Spontaneous ventilation	*1*	9	7.19 ± 0.93	0.73 ± 0.10	1.38 ± 0.16	0.77 ± 0.15	0.085 ± 0.029	1.44 ± 0.16	25.2 ± 1.5	0.446 ± 0.139
Mechanical ventilation, air	*2*	9	4.02 ± 0.56[b]	0.56 ± 0.07	0.94 ± 0.12	0.34 ± 0.08	0.007 ± 0.002[b]	1.01 ± 0.14	16.3 ± 2.9[b]	0.055 ± 0.019[b]
					Acute respiratory alkalosis					
Mechanical hyperventilation, air	*3*	9	3.85 ± 0.65[a]	0.59 ± 0.15	0.89 ± 0.20	0.61 ± 0.16	0.169 ± 0.047	1.14 ± 0.22	28.2 ± 4.7	1.386 ± 0.344[a]
					Acute respiratory acidosis					
Mechanical ventilation, 8% CO_2	*4*	9	3.15 ± 0.49	0.50 ± 0.09	0.45 ± 0.08[c]	0.24 ± 0.05	<0.001[c]	0.58 ± 0.08[c]	12.5 ± 1.7	0.008 ± 0.003[c]
					Chronic respiratory acidosis					
Spontaneous ventilation, 10% CO_2	*5*	8	4.84 ± 1.41	0.43 ± 0.07	0.74 ± 0.23[b]	0.45 ± 0.05[b]	0.028 ± 0.019	1.06 ± 0.26	23.9 ± 3.0	0.104 ± 0.063[b]
Spontaneous ventilation, air	*6*	8	5.67 ± 1.02	0.49 ± 0.06	1.07 ± 0.17	0.60 ± 0.06	0.551 ± 0.100[d]	1.62 ± 0.28	35.2 ± 4.8	3.196 ± 0.571[d]

Values are means ± SE; *n* = no. of animals. Ventilations were on air, acute respiratory alkalosis (mechanical hyperventilation), acute respiratory acidosis (mechanical ventilation on 8% CO_2), chronic respiratory acidosis (spontaneous breathing of 10% CO_2), and during partial recovery from CO_2 adaptation breathing air (treatment *groups 1–6*). GFR, glomerular filtration rate; $U_{Na}V$, urine sodium flow rate; U_KV, urine potassium flow rate; $U_{HCO_3}V$, urine bicarbonate flow rate; FE_{Na}, fractional excretion of sodium; FE_K, fractional excretion of potassium; FE_{HCO_3}, fractional excretion of bicarbonate. [a]$P < 0.05$ comparing *groups 3* vs. *1*. [b]$P < 0.05$ compared with *group 1* (control) by LSD testing after first comparing *groups 1, 2,* and *5* by ANOVA ($P < 0.05$). [c]$P < 0.05$ comparing *groups 4* vs. *2*. [d]$P < 0.05$ comparing *groups 6* vs. *5*. Urine flow and electrolyte excretion rates are expressed per 100 g body wt.

Table 3. *Net bicarbonate reabsorption along the loop of Henle during spontaneous and mechanical ventilation*

Treatment	Group	*n*	Perfusion Rate, nl/min	Perfusion $[HCO_3^-]$, mM	Collection Rate, nl/min	Collected $[HCO_3^-]$, mM	TF/P_{inulin}	J_v, nl/min	HCO_3^- Load, pmol/min^{-1}	$J_{HCO_3^-}$, pmol/min	$FE_{HCO_3^-}$, %
Control											
Spontaneous ventilation, air	*1*	24	19.7 ± 0.3	13.6 ± 0.4	9.5 ± 0.4	9.0 ± 0.6	2.12 ± 0.06	10.2 ± 0.3	267.6 ± 8.3	182.1 ± 12.7	66.6 ± 3.1
Mechanical ventilation, air	*2*	16	21.4 ± 0.5	13.2 ± 0.5	9.3 ± 0.4	7.5 ± 0.5	2.35 ± 0.09	12.1 ± 0.5*	280.8 ± 10.3	210.5 ± 12.2*	74.5 ± 2.4
Acute respiratory alkalosis											
Mechanical hyperventilation, air	*3*	22	19.5 ± 0.3	13.8 ± 0.4	9.1 ± 0.3	7.9 ± 0.5	2.21 ± 0.07	10.7 ± 0.4	273.2 ± 9.5	200.6 ± 11.6	72.5 ± 2.6
Acute respiratory acidosis											
Mechanical ventilation, 8% CO_2	*4*	17	20.2 ± 0.7	13.3 ± 0.4	8.9 ± 0.3	8.8 ± 0.7	2.28 ± 0.07	11.2 ± 0.5	267.6 ± 10.7	189.8 ± 13.6	70.1 ± 2.8
Chronic respiratory acidosis											
Spontaneous ventilation, 10% CO_2	*5*	22	19.4 ± 0.5	12.7 ± 0.4*	8.8 ± 0.3	11.6 ± 0.5*	2.23 ± 0.07	10.6 ± 0.4	245.1 ± 9.0*	143.1 ± 9.7*	57.6 ± 2.3*
Spontaneous ventilation, air	*6*	18	19.7 ± 0.4	13.1 ± 0.2	9.8 ± 0.3†	8.3 ± 0.4†	2.04 ± 0.07	9.9 ± 0.4	256.4 ± 4.5	174.6 ± 6.3†	68.0 ± 2.1†

Values are means ± SE; *n* = no. of tubules. Ventilations were on air, acute respiratory alkalosis (mechanical hyperventilation), acute respiratory acidosis (mechanical ventilation on 8% CO_2), chronic respiratory acidosis (spontaneous breathing of 10% CO_2), and during partial recovery from CO_2 adaptation breathing air (treatment *groups 1–6*). TF/P_{inulin}, ratio of inulin concentrations in collected and perfused fluids; J_v, net fluid flux; J_{HCO_3}, net bicarbonate transport rate. *$P < 0.05$ compared with *group 1* (Control) by LSD testing after first comparing *groups 1, 2,* and *5* by ANOVA ($P < 0.05$); †$P < 0.05$ comparing *groups 6* vs. *5*.

0.05). Acute respiratory alkalosis did not significantly change GFR, sodium fractional excretion (FE_{Na}), or potassium fractional excretion (FE_K), whereas FE_{HCO_3} increased threefold. In acute respiratory acidosis, GFR did not change, but FE_{Na} and FE_{HCO_3} were reduced. In CRA, GFR fell slightly, FE_{Na} and FE_K remained unchanged, and FE_{HCO_3} was low. During the partial recovery phase, CRA (when breathing air) was characterized by a marked increase in bicarbonate excretion (~30-fold), a modest rise in potassium excretion, but no change in GFR.

LOH microperfusion data. Table 3 and Fig. 1 show the microperfusion data obtained in the three different respiratory acid-base states. In the same group of animals, mechanical ventilation on air had no significant effect on J_{HCO_3} (data not shown in Table 3) compared with spontaneous respiration of air (211.8 ± 20.7 vs. 210.5 ± 12.2 pmol/min, $P > 0.05$). In acute respiratory alkalosis, J_{HCO_3} did not change (182.1 ± 12.7 vs. 200.6 ± 11.7 pmol/min, $P > 0.05$). In contrast, FE_{HCO_3} increased significantly in the same experimental animals from 0.45 ± 0.14 to 1.39 ± 0.34% ($P < 0.05$, see Table 2). In acute respiratory acidosis, J_{HCO_3} did not change (210.5 ± 12.2 vs. 189.8 ± 13.6 pmol/min, $P > 0.05$), although the final urinary excretion of bicarbonate was significantly decreased (Table 2). An unexpected finding was that LOH bicarbonate reabsorption was significantly depressed in CRA animals compared with control animals (143.1 ± 9.7 vs. 182.1 ± 12.7 pmol/min, $P < 0.05$), whereas FE_{HCO_3} was low. When these chronically CO_2-adapted animals were switched to breathing air, urinary bicarbonate excretion increased, and J_{HCO_3} rose significantly from 143.1 ± 9.7 to 174.6 ± 6.3 pmol/min ($P < 0.05$). Only

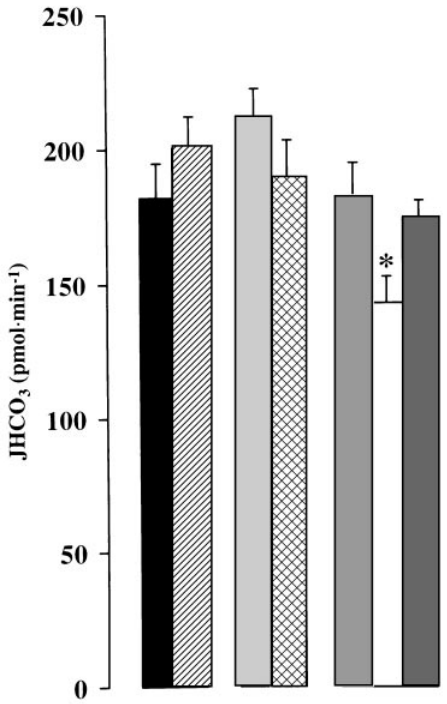

Fig. 1. Effect of spontaneous and mechanical ventilation (air) and respiratory acid-base disturbances on bicarbonate reabsorption (J_{HCO_3}) along the loop of Henle. *Left*: acute respiratory alkalosis (hyperventilation; control, closed bar; acute respiratory alkalosis, hatched bar). *Middle*: acute respiratory acidosis (latticed bar) (ventilation with 8% CO_2; control, shaded bar). *Right*: chronic respiratory acidosis (open bar) (breathing 10% CO_2; control, shaded bar) and partial recovery (breathing air, closed bar). *$P < 0.05$ vs. control, means ± SE.

small and barely significant changes in fluid reabsorption were observed in each treatment group.

Table 4 summarizes the results of further microperfusion experiments designed to assess the contribution of bicarbonate backflux along the LOH to net bicarbonate reabsorption. Experiments were done at 20 nl/min using the standard perfusion solution with and without bicarbonate (*perfusates A* and *B*, respectively). In the absence of bicarbonate in the perfusion solution, there was an influx of bicarbonate of -34.5 ± 4.4 pmol/min into the collected fluid compared with a net efflux of bicarbonate of 210.0 ± 28.1 pmol/min (in the presence of ~15 mM bicarbonate in the perfusate). When experiments were done at two different perfusion rates of 20 and 10 nl/min using a solution containing bicarbonate but designed to minimize net fluid reabsorption (*perfusate C*), net bicarbonate reabsorption was greater at the higher perfusion rate, but fractional reabsorption of bicarbonate was similar at the two perfusion rates. This is consistent with our previously published observations on load and flow dependence of bicarbonate absorption along the LOH (8). However, when compared with the standard perfusion solution containing bicarbonate (*perfusate A*) perfused at 20 nl/min, fractional bicarbonate reabsorption was reduced, indicating that a component of net bicarbonate absorption depends on net fluid reabsorption (solvent drag). When this perfusion solution (*perfusate C*) was bicarbonate free (*perfusate D*), a substantial influx of bicarbonate into the collected fluid occurred at both 20 and 10 nl/min, -47.4 ± 7.8 and -35.9 ± 5.8 pmol/min, respectively. In addition, when acetazolamide was added (*perfusate E*) to abolish carbonic anhydrase-dependent H^+ secretion, a significantly higher influx of bicarbonate into the collected fluid was observed at both perfusion rates, -112.8 ± 5.6 and -72.5 ± 8.8 pmol/min, respectively.

DISCUSSION

In acute respiratory alkalosis and acidosis, there is a rapid (within minutes) change in blood bicarbonate concentration because of blood buffering. Unlike in metabolic acid-base disturbances, in respiratory acid-base imbalance, blood pH and blood bicarbonate concentration change in opposite directions. In respiratory alkalosis, pH increases and blood bicarbonate concentration decreases as P_{CO_2} falls; in respiratory acidosis, pH decreases and blood bicarbonate concentration increases as P_{CO_2} rises. In respiratory alkalosis and acidosis, various methods in different tissues have shown that intracellular pH parallels changes in extracellular pH, although the magnitude of change in pH may be different (1, 5, 24, 41). The changes in plasma potassium concentration within each treatment group, including mechanical ventilation per se, are not easily explained (Table 1). Although metabolic acidosis and alkalosis are associated with hyperkalemia and hypokalemia, respectively, the pattern in respiratory acid-base disturbances is variable and clearly depends on more than changes in systemic pH and intracellular H^+ buffering. Alterations in peripheral sympathetic nerve activity and relative changes in plasma concentrations of norepinephrine and epinephrine seem to be important, although species dependent (27, 39), and may also influence net urinary bicarbonate excretion (33) (Table 2).

Because the rate of H^+ secretion by cells is dependent on the intracellular H^+ concentration, the two main acid-extruding mechanisms, Na^+/H^+ exchange and H^+-ATPase, should be suppressed in respiratory alkalosis and stimulated in respiratory acidosis. There is extensive literature reporting increased activity of both Na^+/H^+ exchange and H^+-ATPase in respiratory acidosis in different epithelia, including the kidney. Proximal tubule membrane vesicle studies and in vitro tubule microperfusion experiments have demonstrated stimulation of apical Na^+/H^+ exchange and basolateral Na^+-$3HCO_3^-$ cotransport activity in respiratory acidosis (25, 40, 45, 46). In the collecting duct and toad bladder, an increase in P_{CO_2} has been shown to stimulate H^+-ATPase activity (36, 38). This has also been confirmed in the collecting duct by immunocytochemical studies (4). Assays of renal H^+-ATPase and H^+-K^+-ATPase activities following respiratory acid-base changes show them to be increased in respiratory acidosis after 24 h and decreased in respiratory alkalosis after 6 h (17). There are no reports of the effect of

Table 4. *Assessment of contribution of bicarbonate backflux to net bicarbonate reabsorption*

Perfusate	*n*	Perfusion Rate, nl/min	Perfusate $[HCO_3^-]$, mM	Collection Rate, nl/min	Collected $[HCO_3^-]$, mM	TF/P_{inulin}	J_V, nl/min	HCO_3^- Load, pmol/min	$J_{HCO_3^-}$, pmol/min	$FE_{HCO_3^-}$, %
A	10	18.9 ± 0.8	14.0 ± 1.0	10.4 ± 0.4	5.5 ± 0.7	1.83 ± 0.06	8.5 ± 0.6	268.2 ± 27.5	210.0 ± 28.1	76.7 ± 4.1
B	20	18.7 ± 0.4	0	9.6 ± 0.4	3.8 ± 0.5	2.03 ± 0.11	9.1 ± 0.5	0	−34.5 ± 4.4	
C	15	20.1 ± 0.5	9.9 ± 0.3	17.9 ± 0.9	5.3 ± 0.5	1.16 ± 0.04	2.3 ± 0.6	199.6 ± 10.1	106.1 ± 10.9	52.4 ± 4.0
D	8	20.1 ± 0.5	0	17.3 ± 0.6	2.7 ± 0.4	1.17 ± 0.04	2.8 ± 0.7	0	−47.4 ± 7.8	
E	6	20.4 ± 1.3	0	18.1 ± 0.8	6.3 ± 0.4	1.13 ± 0.03	2.3 ± 0.7	0	−112.8 ± 5.6	
C	10	9.6 ± 0.3	9.7 ± 0.3	8.4 ± 0.3	5.3 ± 0.6	1.15 ± 0.04	1.2 ± 0.3	93.7 ± 4.1	48.5 ± 3.4	53.0 ± 4.5
D	7	9.6 ± 0.5	0	8.6 ± 0.5	4.3 ± 0.8	1.12 ± 0.04	1.0 ± 0.3	0	−35.9 ± 5.8	
E	10	10.1 ± 0.5	0	9.5 ± 0.4	7.5 ± 0.8	1.06 ± 0.03	0.6 ± 0.3	0	−72.5 ± 8.8	

Values are means ± SE; *n* = no. of tubules. Perfusates are loop of Henle (LOH) perfusions with standard perfusion solution in the presence and absence of bicarbonate (*perfusates A* and *B*, respectively) perfused at 20 nl/min, LOH perfusions with a low-NaCl perfusate containing mannitol (isotonic) with and without bicarbonate (*perfusates C* and *D*, respectively), and bicarbonate-free plus acetazolamide (2×10^{-4} M, *perfusate E*) perfused at 20 and 10 nl/min, respectively.

more acute hypocapnea on H^+-ATPase activity or Na^+/H^+ exchange.

When tubular bicarbonate load is fixed and is independent of GFR, renal tubular absorption of bicarbonate depends on luminal H^+ secretion (34); an increase in H^+ secretion will enhance and a decrease will depress net bicarbonate reabsorption. Therefore, we would expect increased bicarbonate reabsorption in respiratory acidosis and decreased reabsorption in respiratory alkalosis. However, another important variable to consider is the peritubular capillary blood bicarbonate concentration and the transepithelial bicarbonate concentration gradient. This could influence net bicarbonate reabsorption by affecting tubule cell bicarbonate exit (Na^+-$3HCO_3^-$ cotransport) and paracellular bicarbonate flux. In metabolic acid-base disturbances, these changes are in parallel and therefore additive: in metabolic acidosis, increased H^+ secretion is associated with a reduced blood bicarbonate concentration and reduced bicarbonate backflux (from blood to lumen); in metabolic alkalosis, decreased H^+ secretion is associated with a raised blood bicarbonate concentration and increased bicarbonate backflux. The situation is different in respiratory acid-base disorders, in which changes in P_{CO_2} parallel tubule cell H^+ secretion, and blood bicarbonate concentration also mirrors P_{CO_2}, i.e., increased blood P_{CO_2} and bicarbonate in acidosis and decreased blood P_{CO_2} and bicarbonate in alkalosis. Thus an increase in peritubular capillary blood bicarbonate concentration may oppose and a decrease in blood bicarbonate concentration favor net bicarbonate reabsorption. Such an effect is likely to be more important in leaky epithelia, like the proximal tubule, than in tight epithelia, like the distal nephron. Because the LOH is an intermediate segment of mixed epithelia, the influence of changes in blood bicarbonate concentration on net bicarbonate transport is less clear.

We observed no change in net bicarbonate absorption in the LOH in either acute respiratory alkalosis or acidosis. In the proximal tubule, earlier in vitro microperfusion and in vivo micropuncture studies reported inhibition of net bicarbonate absorption in alkalosis and slight stimulation in respiratory acidosis (13, 23). However, Ullrich et al. (42) and Zeidel and Seifter (46) found no change in proximal tubule acidification in respiratory acidosis. A more recent in vivo study by Santella et al. (35) also found no effect under these conditions when changes in the bicarbonate filtered load were taken into account. These authors concluded that the major determinant of net bicarbonate transport in this part of the nephron is the bicarbonate load. Along the LOH, we have also shown that bicarbonate reabsorption is load dependent (8). To control for this, we did our microperfusion experiments using a fixed load of bicarbonate. Therefore, any changes in net bicarbonate transport along the LOH must reflect alterations in the activity of the acid-base transporters and the degree of bicarbonate backflux. The two main luminal acidifying mechanisms along the LOH are the Na^+/H^+ exchanger and the H^+-ATPase (8). As already mentioned, these transporters are stimulated by a rise in P_{CO_2}, and they are probably inhibited by a fall in P_{CO_2}. Because we found no change in J_{HCO_3} in acute respiratory alkalosis and acidosis, this suggests that any change in active transport activity is countered by an opposite change in bicarbonate backflux, which in turn depends on the blood-to-lumen bicarbonate concentration gradient, i.e., an increased gradient in respiratory acidosis and a decreased gradient in respiratory alkalosis. This interpretation is strengthened by our observation that, in chronic respiratory acidosis, in which there was a steeper blood-to-lumen bicarbonate gradient than in acute respiratory acidosis, there was inhibition of net bicarbonate transport. In addition, on switching these acidotic chronically CO_2-adapted animals from 10% CO_2 to air, there was a rapid increase in J_{HCO_3} to control values (see Fig. 1). Although the blood-to-lumen bicarbonate concentration gradient decreased and therefore the amount of bicarbonate backflux, the blood bicarbonate concentration was still significantly elevated. In chronic respiratory acidosis, it has been shown that stimulated Na^+/H^+ exchanger activity remains increased for up to 24 h after withdrawal of the elevated CO_2 (45). Thus it is possible that some of the rebound in J_{HCO_3} that we observed (Fig. 1) is also due to a persistent enhancement of Na^+/H^+ exchanger activity in the presence of a smaller blood-to-lumen bicarbonate gradient.

The renal clearance data are similar to earlier studies showing an initial increase in bicarbonate excretion in acute respiratory alkalosis and a decrease in acute

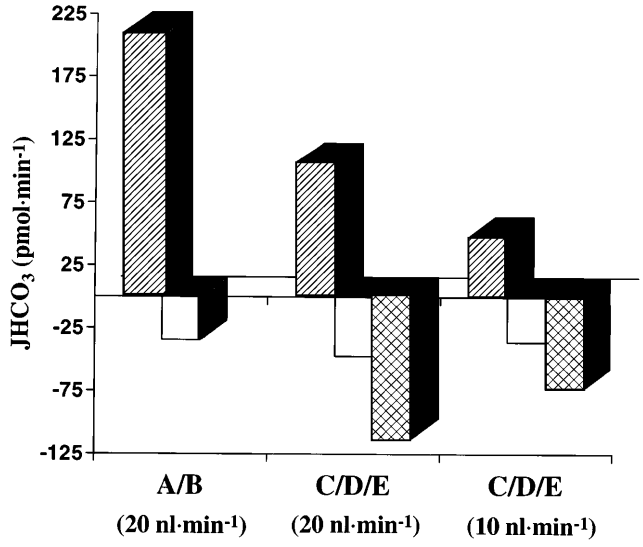

Fig. 2. *Left*: effect of removing bicarbonate from the standard perfusate delivered at 20 nl/min on net bicarbonate flux (J_{HCO_3}) along the LOH: *perfusate A*, with bicarbonate (hatched bar); *perfusate B*, without bicarbonate (open bar). *Middle*: effect of removing bicarbonate and minimizing net fluid reabsorption and abolishing active H^+ secretion on J_{HCO_3} during perfusion at 20 nl/min: *perfusate C*, control low NaCl with bicarbonate (hatched bar); *perfusate D*, low NaCl without bicarbonate (open bar); *perfusate E*, low NaCl without bicarbonate and with acetazolamide (latticed bar). *Right*: effect of removing bicarbonate and minimizing net fluid reabsorption and abolishing active H^+ secretion on J_{HCO_3} during perfusion at 10 nl/min; perfusate composition is as in *middle* group. Mean values only are shown (see Table 4).

and chronic respiratory acidosis (37). However, a striking observation was the marked increase in urinary bicarbonate excretion following the switch from breathing high CO_2 to air in chronically CO_2-adapted animals. This cannot be explained by a change in GFR or bicarbonate reabsorption along the proximal nephron, including the LOH, but must be due to a rapid inhibition of bicarbonate absorption beyond the LOH. It seems likely that this is mediated by a change in H^+-ATPase, which is known to be regulated by P_{CO_2} and blood bicarbonate concentration (17). It has been shown that luminal membrane insertion and withdrawal of this transporter are fast and sensitive to changes in P_{CO_2} (36). In addition, there might also be a relative increase in apical Cl^-/HCO_3^- exchange activity and bicarbonate secretion.

To confirm the importance of bicarbonate backflux in the LOH, we did further experiments to assess whole segment transepithelial permeability to bicarbonate. Although these experiments do not discriminate among any differences in bicarbonate permeability of the various nephron segments that make up the LOH, they do take account of the corticomedullary solute gradients in vivo. Bicarbonate permeability has been estimated in more homogeneous nephron segments of the proximal and distal tubules in vivo, and the results show a decrease in permeability from proximal to distal nephron (11, 12, 20, 29, 44). As illustrated in Fig. 2, which shows only the mean and directional changes in net bicarbonate flux, our observations under conditions of minimal net fluid reabsorption and inhibition of active H^+ secretion along the LOH in vivo demonstrate that passive bicarbonate backflux can be up to 50% of active bicarbonate reabsorption. In keeping with these findings is the observation of Byers at al. (7), who perfused the LOH in vivo with a bicarbonate-free solution and found an accumulation of 4 mM bicarbonate in the collected fluid, a value almost identical with our own (see Table 4). Moreover, in our previous study of LOH perfusion in which there was a large blood-to-lumen bicarbonate gradient, we found that inhibition of luminal Na^+/H^+ exchange and H^+-ATPase changed net bicarbonate flux from reabsorption to secretion (10). Under normal acid-base conditions in vivo, a corticomedullary gradient for interstitial bicarbonate concentration (measured as total CO_2) exists and favors net bicarbonate transfer from vasa recta to juxtamedullary LOHs (18), which could facilitate adaptation to an alkali load (19).

In conclusion, our results show that, when the LOH is perfused in vivo with a fixed bicarbonate load, changes in respiratory acid-base balance do not alter net bicarbonate reabsorption. This apparent lack of effect of respiratory alkalosis or acidosis on LOH bicarbonate reabsorption could be explained by concomitant changes in the blood-to-lumen bicarbonate concentration gradient offsetting any inhibition or stimulation of H^+ secretion induced by a fall or rise in P_{CO_2}, respectively. In chronic respiratory acidosis, there was indirect evidence for an underlying stimulation of LOH acidification that was opposed by the associated rise in blood bicarbonate concentration and blood-to-lumen bicarbonate gradient. Finally, the importance of bicarbonate backflux along the LOH was confirmed by additional experiments, the results of which demonstrate that in vivo bicarbonate permeability and passive flux of bicarbonate are major determinants of net bicarbonate transport, and this is not always considered under in vitro experimental conditions.

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