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M G Cogan

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Research Article

The hyperbicarbonatemia of chronic respiratory acidosis might be maintained by a reduction in filtration rate or an enhancement of tubular bicarbonate reabsorption. To investigate this question, 12 Munich-Wistar rats were exposed to a 10% CO2 atmosphere for 6-8 d. Chronic respiratory acidosis developed, with arterial pH 7.30 +/- 0.01, partial pressure of CO2 (pCO2) 80 +/- 2 mmHg, and total CO2 concentration 45 +/- 1 mM. Single nephron glomerular filtration rate was normal (42 +/- 1 nl/min). Chronic hypercapnia caused absolute proximal reabsorption to be significantly stimulated (1,449 +/- 26 pmol/min) as compared with reabsorption previously observed in normal animals (1,075 +/- 74 pmol/min) or in animals subjected to acute hypercapnia (1,200 +/- 59 pmol/min). This is the first demonstration that proximal bicarbonate reabsorption can be stimulated above normal euvolemic values. When eight animals were subsequently allowed to return toward a normocapnic state (arterial pCO2 46 +/- 1 mmHg) over the course of 1-1.5 h, bicarbonate reabsorption was still significantly higher (1,211 +/- 34 pmol/min) than in similarly alkalotic, normocapnic control groups (994 +/- 45 pmol/min). In conclusion, chronic, but not acute, hypercapnia stimulates absolute proximal bicarbonate reabsorption to exceed the level found in normal euvolemic rats.

Chronic Hypercapnia Stimulates Proximal Bicarbonate Reabsorption in the Rat

Martin G. Cogan
Cardiovascular Research Institute and Department of Medicine, University of California, San Francisco, California 94143

Abstract. The hyperbicarbonatemia of chronic respiratory acidosis might be maintained by a reduction in filtration rate or an enhancement of tubular bicarbonate reabsorption. To investigate this question, 12 Munich-Wistar rats were exposed to a 10% CO_2 atmosphere for 6–8 d. Chronic respiratory acidosis developed, with arterial pH 7.30±0.01, partial pressure of CO_2 (pCO_2) 80±2 mmHg, and total CO_2 concentration 45±1 mM. Single nephron glomerular filtration rate was normal (42±1 nl/min). Chronic hypercapnia caused absolute proximal reabsorption to be significantly stimulated (1,449±26 pmol/min) as compared with reabsorption previously observed in normal animals (1,075±74 pmol/min) or in animals subjected to acute hypercapnia (1,200±59 pmol/min). This is the first demonstration that proximal bicarbonate reabsorption can be stimulated above normal euvolemic values. When eight animals were subsequently allowed to return toward a normocapnic state (arterial pCO_2 46±1 mmHg) over the course of 1–1.5 h, bicarbonate reabsorption was still significantly higher (1,211±34 pmol/min) than in similarly alkalotic, normocapnic control groups (994±45 pmol/min). In conclusion, chronic, but not acute, hypercapnia stimulates absolute proximal bicarbonate reabsorption to exceed the level found in normal euvolemic rats.

Introduction

The high plasma bicarbonate concentration found in chronic respiratory acidosis might be maintained in two ways. First, glomerular filtration might fall as the plasma bicarbonate concentration rises, thus attenuating or preventing the increase in filtered bicarbonate load. Relatively normal rates of tubular hydrogen ion secretion would then suffice to prevent bicarbonate from appearing in the urine. This mechanism has been proposed to be the one that maintains the other major hyperbicarbonatemic acid-base disorder, chronic metabolic alkalosis (1). On the other hand, if glomerular filtration remained normal, enhanced tubular bicarbonate reabsorption, predominantly in the proximal nephron, would be needed to prevent bicarbonaturia.

Measurement of absolute rates of bicarbonate filtration and proximal reabsorption needed to differentiate these two pathophysiological mechanisms have not been reported to date. In previous free-flow micropuncture studies fractional proximal bicarbonate reabsorption in chronic hypercapnia appeared normal, a finding that does not allow discrimination between the two alternatives (2). Split-droplet studies during chronic hypercapnia have found a relatively normal rate of proximal acidification (3), which would support the first process discussed above (reduced filtration rate with normal bicarbonate reabsorption). Acute hypercapnia has also been found to have only a small effect on free-flow proximal bicarbonate reabsorption (4). In fact, no factor has yet been reported to reliably increase proximal bicarbonate reabsorption above the mean level found in the normal euvolemic state (1, 5).

Thus, the purpose of the present studies was to measure absolute rates of bicarbonate filtration and reabsorption in rats rendered chronically hypercapnic.

Methods

Protocols. To induce chronic respiratory acidosis, 12 Munich-Wistar rats were exposed to 10% CO_2 (air balance) in an environmental chamber for 6–8 d. Gas samples from the chamber were periodically measured to ensure that the 10% CO_2 atmosphere was constant. Rats were allowed chow diet and distilled water ad lib. while in the chamber. On the day of micropuncture study, the animal was rapidly weighed (mean weight was 184±4 g), injected intraperitoneally with Inactin (100 mg/kg), and immediately placed into a holding chamber flooded with the same 10% CO_2 gas mixture. When anesthetized, the rat was removed from the holding chamber, a tracheostomy and intubation were performed as rapidly as possible (≤2 min), and 10% CO_2 was again administered via a T-tube apparatus, as previously described (4).

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Address reprint requests to Dr. Cogan, 1065 HSE, University of California, San Francisco, CA 94143.

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The rest of the surgical preparation and free-flow micropuncture techniques were performed as previously outlined (5). Surgically induced plasma volume losses, assumed to be similar to previously established values (1.3% body weight), and whole blood losses from sampling were quantitatively replaced (5). Donors for plasma and whole blood were also maintained in the environmental chamber with 10% CO_2 for 6–8 d.

After the first period of micropuncture, eight animals were extubated and allowed to breathe room air to allow arterial partial pressure of CO_2 (pCO_2)[1] to decrease toward normal. After a 30–60-min equilibration, the animals were again studied by micropuncture during this post-hypercapnic period. The time between extubation and the end of the second micropuncture period was ~1–1.5 h.

A separate group of 15 animals was rendered acutely alkalotic to serve as normocapnic controls for the post-hypercapnic animals. A 10% body weight bicarbonate solution ($NaHCO_3$ 200 mM plus KCl 25 mM) was infused over 1 h, and followed by a maintenance infusion of 0.1 ml/min. Micropuncture began after an equilibration of 30 min. Tubular chloride concentrations were not measured in this group.

Analysis and calculations. Plasma, urine, and tubular fluid measurements were made as previously described (1, 4, 5). [^{3}H]inulin was measured by scintillation counting, total CO_2 by microcalorimetry (6), and chloride by the method of Ramsay (7). Calculations of filtration and reabsorptive rates were also as previous outlined (1, 4, 5). Results are presented as mean±SEM, and significance was assessed by the paired or unpaired *t* test as appropriate.

Results

Exposure to 10% CO_2 for 6–8 d resulted in chronic respiratory acidosis. Arterial measurements were: pH 7.30±0.01; pCO_2, 80±2 mmHg; plasma total CO_2 concentration, 45±1 mM; and plasma chloride concentration, 93±1 meq/liter. Other blood measurements and urinary excretion data, shown in Table I, were generally similar to those previously reported for normal euvolemic rats (5, 8). Kidney weight was 0.90±0.04 g.

Micropuncture results are shown in Table II. Single nephron glomerular filtration rate (SNGFR) (42.4±0.7 nl/min) was normal for rats of this size (5, 8). The elevated total CO_2 concentration in Bowman's space (47.9±0.9 mM) was therefore associated with a high filtered total CO_2 load (2,025±47 pmol/min). Chronic hypercapnia caused absolute proximal total CO_2 reabsorption to be stimulated to 1,449±26 pmol/min, a value 35% higher than that of a normal euvolemic animal (1,075±74 pmol/min, $P < 0.001$) (5).

Bicarbonate reabsorption during chronic hypercapnia was also higher than values previously reported after acute exposure to 10% CO_2 (4). The comparison of micropuncture data during chronic and acute hypercapnia is shown in Table III. In chronic hypercapnia, total CO_2 reabsorption was 23% higher than when acute hypercapnia was superimposed on chronic metabolic alkalosis, despite there being only a slight difference in the logarithmic mean luminal total CO_2 concentration, an important determinant of proximal acidification (5, 9, 10). Total CO_2 reabsorption was also 21% higher during chronic hypercapnia than when acute hypercapnia was superimposed on acute metabolic alkalosis. In this case, the acutely hypercapnic group had a higher SNGFR and mean luminal total CO_2 concentration, factors which, if anything, should accelerate acidification (9, 10). The stimulation of bicarbonate reabsorption over mean normal levels in the chronically hypercapnic group but not in the combined acutely hypercapnic groups is illustrated in Fig. 1.

Absolute chloride reabsorption during chronic respiratory acidosis (Table II) was slightly reduced from normal values, possibly as a result of the hypochloremic state (1), whereas absolute water reabsorption was approximately the same as in normal euvolemic animals (5, 8).

When eight of the animals were extubated and allowed to breathe room air, a stable post-hypercapnic metabolic alkalosis developed, with arterial pH of 7.50±0.01 and pCO_2 of 46±1 mmHg (Table I). Plasma total CO_2 concentration fell to 37±1 mM, associated with bicarbonaturia. SNGFR was stable (Table II). The resulting decline in filtered total CO_2 load (1,665±68 pmol/min) was associated with a fall in absolute proximal total CO_2 reabsorption to 1,211±34 pmol/min. However, this

Table I. Blood Composition and Urinary Excretion Rates in Chronic Hypercapnia and Post-Hypercapnia

	Chronic hypercapnia	Post-hypercapnia	*P*
Blood			
Arterial			
pH	7.30±0.01	7.50±0.01	<0.001
pCO_2 (*mmHg*)	80±2	46±1	<0.001
Blood pressure (*mmHg*)	130±4	114±3	<0.005
Hematocrit (*vol %*)	47.5±0.7	46.5±0.5	<0.025
Plasma			
[Na^+] (*meq/liter*)	149±1	146±1	<0.01
[K^+] (*meq/liter*)	3.9±0.1	3.9±0.1	NS
[Total CO_2] (*mM*)	45±1	37±1	<0.001
[Cl^-] (*meq/liter*)	93±1	96±1	<0.05
[Protein] (*g/dl*)	5.2±0.1	5.0±0.1	<0.25
Urine			
Glomerular filtration rate (*ml/min·g kwt*)	1.0±0.1	1.0±0.1	NS
Urinary excretion			
Na^+ (*μeq/min*)	0.2±0.1	0.2±0.1	NS
K^+ (*μeq/min*)	0.5±0.1	1.0±0.1	<0.01
Total CO_2 (*μmol/min*)	0.05±0.02	0.4±0.1	<0.05
Cl^- (*μeq/min*)	0.5±0.1	1.0±0.4	NS
Volume (*μl/min*)	2.9±0.4	2.4±0.2	NS

kwt, kidney weight; NS, not significant.

1. *Abbreviations used in this paper:* pCO_2, partial pressure of CO_2; SNGFR, single nephron glomerular filtration rate.

Table II. Proximal Filtration and Reabsorption during Chronic Hypercapnia and Post-Hypercapnia

		Bowman's space		End-proximal			Absolute proximal reabsorption			Fractional proximal reabsorption		
Group	SNGFR*	$[tCO_2]$	[Cl]	$[tCO_2]$	[Cl]	V	tCO_2	Cl	H_2O	tCO_2	Cl	H_2O
	nl/min	*mM*	*meq/liter*	*mM*	*meq/liter*	*nl/min*	*pmol/min*	*peq/min*	*nl/min*			
Chronic hypercapnia (n = 12)	42.4±0.7	47.9±0.9	102.9±2.4	23.5±1.2	126.0±2.4	24.2±1.0	1,449±26	1,322±131	18.1±0.6	0.72±0.02	0.30±0.02	0.43±0.02
Post-hypercapnia (n = 8)	40.4±1.3	41.2±0.9	106.4±2.3	18.5±1.4	130.3±2.4	24.2±1.2	1,211±34	1,140±141	16.2±0.3	0.73±0.02	0.27±0.03	0.40±0.01
P‡	NS	<0.001	NS	<0.05	NS	NS	<0.01	NS	NS	NS	NS	NS
Acute metabolic alkalosis (n = 15)	46.3±2.2	45.1±1.0	—	35.5±1.3	—	30.1±1.7	1,015±49	—	16.2±0.8	0.49±0.01	—	0.35±0.01
P§	NS	<0.05	—	<0.001	—	<0.05	<0.01	—	NS	<0.001	—	<0.025

Means±SEM. * tCO_2, total CO_2, V, end-proximal flow rate; NS, not significant. ‡ P value comparing chronic hypercapnia with post-hypercapnia. § P value comparing post-hypercapnia with acute metabolic alkalosis.

Table III. Proximal Bicarbonate Reabsorption during Hypercapnic States

Group	Bowman's space [total CO_2]	SNGFR	Filtered total CO_2 load	End-proximal [total CO_2]	Log mean [total CO_2]	Absolute proximal total CO_2 reabsorption
	mM	*nl/min*	*pmol/min*	*mM*	*mM*	*pmol/min*
Chronic hypercapnia (n = 12)	47.9±0.9	42.4±0.7	2,025±47	23.5±1.2	34±1	1,449±26
Acute hypercapnia*						
+ chronic metabolic alkalosis (n = 10)	50.4±1.7	27.9±1.7	1,385±98	15.5±1.1	30±1	1,176±77
P‡	NS	<0.001	<0.001	<0.001	<0.05	<0.005
+ Acute metabolic alkalosis (n = 7)	58.6±1.5	50.1±1.5	2,951±249	49.0±1.5	54±1	1,234±97
P‡	<0.001	<0.025	<0.001	<0.001	<0.001	<0.025

* From reference 4. ‡ As compared with chronic hypercapnia.

value for total CO_2 reabsorption in the post-hypercapnic period was still significantly higher than in a previous report on a group of 22 animals with chronic metabolic alkalosis (981±49 pmol/min, $P < 0.025$) that had both a similar arterial pCO_2 (43±1 mmHg) and a mean luminal total CO_2 concentration (32 mM) (1). The post-hypercapnic value was also higher than in the 15 animals in this study with acute metabolic alkalosis (1,015±49 pmol/min, $P < 0.01$), as shown in Table II. This latter group had a slightly lower arterial pCO_2 (39±1 mmHg) but a higher SNGFR (46±2 nl/min) and mean luminal total CO_2 concentration (40 mM). Thus, as illustrated in Fig. 2, stimulation of proximal bicarbonate reabsorption by chronic hypercapnia appeared to persist, at least to some degree, after inhalation of CO_2 was terminated.

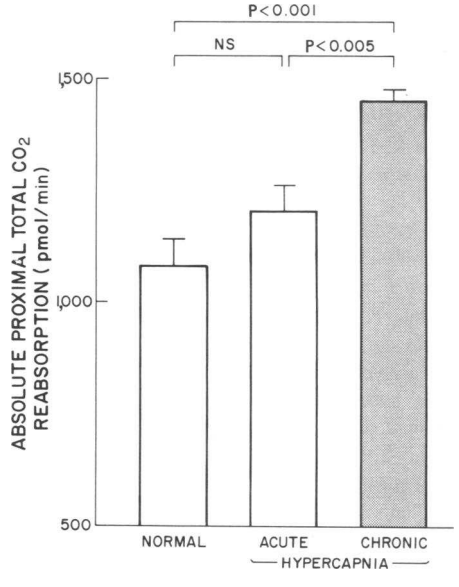

Figure 1. Comparison of absolute proximal bicarbonate reabsorption in rats with chronic hypercapnia with reabsorption in normal euvolemic rats (from reference 5) and with reabsorption in combined groups of rats with acute hypercapnia superimposed on chronic or acute metabolic alkalosis (from reference 4).

Discussion

It has long been known that several days are required for the full increase in renal bicarbonate reabsorption due to a sustained

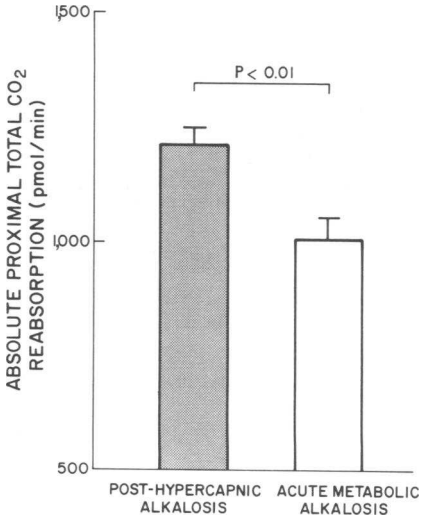

Figure 2. Comparison of absolute proximal bicarbonate reabsorption in rats with post-hypercapnic alkalosis with reabsorption in rats with acute metabolic alkalosis (no pre-existing hypercapnic stimulus).

increment in arterial pCO_2 (11), irrespective of sodium intake (12), bicarbonate availability and rate of net acid excretion (13), potassium stores (14), or mineralocorticoid activity (15). The present studies demonstrate that this renal adaptive response is mediated not by altered hemodynamics (i.e., by a reduction in glomerular filtration rate) but rather by increased bicarbonate transport in the superficial proximal convoluted tubule. A simultaneous adaptation in bicarbonate reabsorption by sites distal to the superficial proximal convoluted tubule and/or by juxtamedullary nephrons is also possible. That such nephron segments contribute to the adaptive response is supported by the finding that when the arterial pCO_2 was allowed to return toward normal in the second period, bicarbonaturia appeared despite the fact that bicarbonate delivery out of the superficial proximal tubule was lower (454±46 post-hypercapnia as compared with 577±45 pmol/min during chronic hypercapnia).

The mechanism underlying this stimulation of proximal bicarbonate reabsorption during chronic hypercapnia might involve either a primary increase in Na^+/H^+ exchanger activity and/or in bicarbonate exit. Rector has argued for the former based on the time course of changes in ammonium excretion induced by hypercapnia (16). An increase in antiporter activity caused by chronic metabolic acidosis has been recently demonstrated (17–19). Studies using vesicle preparations or intracellular microelectrodes will be necessary to clarify whether a similar mechanism is induced by chronic respiratory acidosis.

The augmentation of proximal bicarbonate reabsorption persisted, to some degree, for as long as 1.5 h after the discontinuation of CO_2 inhalation. The possibility that cellular transport processes might have a "memory" of pre-existing conditions has been previously proposed for acidification in the distal tubule after hypocapnia (20), metabolic acid-base disturbances (21), or dietary manipulations (22). Changes in glycodiazine transport after acid-base disorders (3) and in volume adsorption measured in vitro after partial renal ablation in vivo (23) have also been documented in the proximal convoluted tubule. Such a memory effect in the proximal tubule after normalization of a high CO_2 tension might be at least partially responsible for maintaining post-hypercapnic metabolic alkalosis, previously attributed solely to extracellular volume depletion (13).

In conclusion, chronic but not acute hypercapnia increased absolute proximal bicarbonate reabsorption. This is the first demonstration that proximal bicarbonate reabsorption can be stimulated above the mean level found in a normal, euvolemic animal.

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