Relationship of urinary and blood carbon dioxide tension during hypercapnia in the rat. Its significance in the evaluation of collecting duct hydrogen ion secretion.

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Research Article

This study was designed to establish the relationship between urinary pCO2 and systemic blood pCO2 during acute hypercapnia and to investigate the significance of this relationship to collecting duct hydrogen ion (H+) secretion when the urine is acid and when it is highly alkaline. In rats excreting a highly alkaline urine, an acute increase in blood pCO2 (from 42 +/- 0.8 to 87 +/- 0.8 mmHg) resulted in a significant fall in urine minus blood (U-B) pCO2 (from 31 +/- 2.0 to 16 +/- 4.2 mmHg, P less than 0.005), a finding which could be interpreted to indicate inhibition of collecting duct H+ secretion by hypercapnia. The urinary pCO2 of rats with hypercapnia, unlike that of normocapnic controls, was significantly lower than that of blood when the urine was acid (58 +/- 6.3 and 86 +/- 1.7 mmHg, P less than 0.001) and when it was alkalinized in the face of accelerated carbonic acid dehydration by infusion of carbonic anhydrase (78 +/- 2.7 and 87 +/- 1.8 mmHg, P less than 0.02). The finding of a urinary pCO2 lower than systemic blood pCO2 during hypercapnia suggested that the urine pCO2 prevailing before bicarbonate loading should be known and the blood pCO2 kept constant to evaluate collecting duct H+ secretion using the urinary pCO2 technique. In experiments performed under these conditions, […]

Relationship of Urinary and Blood Carbon Dioxide Tension during Hypercapnia in the Rat

Its Significance in the Evaluation of Collecting Duct Hydrogen Ion Secretion

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Abstract

This study was designed to establish the relationship between urinary pCO_2 and systemic blood pCO_2 during acute hypercapnia and to investigate the significance of this relationship to collecting duct hydrogen ion (H^+) secretion when the urine is acid and when it is highly alkaline. In rats excreting a highly alkaline urine, an acute increase in blood pCO_2 (from 42±0.8 to 87±0.8 mmHg) resulted in a significant fall in urine minus blood (U−B) pCO_2 (from 31±2.0 to 16±4.2 mmHg, $P < 0.005$), a finding which could be interpreted to indicate inhibition of collecting duct H^+ secretion by hypercapnia. The urinary pCO_2 of rats with hypercapnia, unlike that of normocapnic controls, was significantly lower than that of blood when the urine was acid (58±6.3 and 86±1.7 mmHg, $P < 0.001$) and when it was alkalinized in the face of accelerated carbonic acid dehydration by infusion of carbonic anhydrase (78±2.7 and 87±1.8 mmHg, $P < 0.02$). The finding of a urinary pCO_2 lower than systemic blood pCO_2 during hypercapnia suggested that the urine pCO_2 prevailing before bicarbonate loading should be known and the blood pCO_2 kept constant to evaluate collecting duct H^+ secretion using the urinary pCO_2 technique.

In experiments performed under these conditions, sodium bicarbonate infusion resulted in an increment in urinary pCO_2 (i.e., a ΔpCO_2) which was comparable in hypercapnic and normocapnic rats (40±7.2 and 42±4.6 mmHg, respectively) that were alkalemic (blood pH 7.53±0.02 and 7.69±0.01, respectively). The U−B pCO_2, however, was again lower in hypercapnic than in normocapnic rats (15±4.0 and 39±2.5 mmHg, respectively, $P < 0.001$). In hypercapnic rats in which blood pH during bicarbonate infusion was not allowed to become alkalemic (7.38±0.01), the ΔpCO_2 was higher than that of normocapnic rats which were alkalemic (70±5.6 and 42±4.6 mmHg, respectively, $P < 0.005$) while the U−B pCO_2 was about the same (39±3.7 and 39±2.5 mmHg). We further examined urine pCO_2 generation by measuring the difference between the urine pCO_2 of a highly alkaline urine not containing carbonic anhydrase and that of an equally alkaline urine containing this enzyme. Carbonic anhydrase infusion to hypercapnic rats that were not alkalemic resulted in a fall in urine pCO_2 (from 122±5.7 to 77±2.2 mmHg) which was greater ($P < 0.02$) than that seen in alkalemic normocapnic controls (from 73±1.9 to 43±1.3 mmHg) with a comparable urine bicarbonate concentration and urine nonbicarbonate buffer capacity. CO_2 generation, therefore, from collecting duct H^+ secretion and titration of bicarbonate, was higher in hypercapnic rats than in normocapnic controls.

We conclude that in rats with acute hypercapnia, the U−B pCO_2 achieved during bicarbonate loading greatly underestimates collecting duct H^+ secretion because it is artificially influenced by systemic blood pCO_2. The ΔpCO_2 is a better qualitative index of collecting duct H^+ secretion than the U−B pCO_2, because it is not artificially influenced by systemic blood pCO_2 and it takes into account the urine pCO_2 prevailing before bicarbonate loading.

Introduction

The carbon dioxide tension (pCO_2) of a highly alkaline urine reaches a value substantially higher than that of systemic blood (1–8). Pitts and Lotspeich (1) proposed that the existence of an appreciable gradient between urine and blood pCO_2 was the consequence of distal H^+ secretion into bicarbonate-rich tubular urine in the face of delayed carbonic acid dehydration. They reasoned that, in the absence of carbonic anhydrase in the lumen of the distal nephron, carbonic acid would not completely dehydrate to carbon dioxide during the time that tubular fluid is in transit through the distal nephron. Completion of carbonic acid dehydration in the renal pelvis would ultimately increase urinary pCO_2 because the surface to volume relationship in this region is not favorable for CO_2 back diffusion (1).

It is now known from recent direct intrarenal pCO_2 determinations that during alkalinization of the urine a near maximal urinary pCO_2 is achieved in the papillary collecting duct (9, 10). That the rise in urinary pCO_2 is an intrarenal rather than a postpapillary event, however, does not invalidate the classic notion that distal H^+ secretion is largely responsible for the high urinary pCO_2 achieved in a highly alkaline urine. Although other explanations have been offered to account for the development of a urine to blood pCO_2 gradient (3, 6, 7, 11–17), it is generally accepted that distal H^+ secretion largely accounts for this phenomenon (18–29). The recent finding of a significant disequilibrium pH in the papillary collecting duct under conditions of bicarbonate loading has been proposed as proof of the notion that H^+ secretion by the collecting duct is the most likely determinant of the urine minus blood (U−B)[1] pCO_2 gradient observed in an alkaline urine (9).

The U−B pCO_2 gradient achieved in response to bicarbon-

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1. *Abbreviations used in this paper:* GFR, glomerular filtration rate; U−B, urine minus blood.

ate loading, however, may not be the ideal way to express the relationship between urinary pCO_2 generation and distal H^+ secretion (30). The use of the U−B pCO_2 as a marker of distal H^+ secretion assumes that blood pCO_2 and urine pCO_2 are about the same before alkalinization of the urine. There are situations, however, where the urinary pCO_2 can be lower than blood pCO_2 as in metabolic and respiratory acidosis (3, 6, 31–33). It also assumes that during bicarbonate loading medullary blood pCO_2 is similar to that of systemic blood. The latter assumption was supported by the findings of Uhlich et al. (34), which showed that during bicarbonate loading vasa recta blood pCO_2 was not substantially different from that prevailing in systemic blood (as determined by renal artery pCO_2). Using a recently developed pCO_2 microelectrode (35–39), DuBose (9), however, found that during bicarbonate loading the pCO_2 of the papillary collecting duct is equal to that of its adjacent vasa recta but is markedly higher than systemic blood pCO_2. He suggested a role for trapping of CO_2 in the medullary countercurrent system to explain the similar values for pCO_2 in the papillary collecting duct and its adjacent vasa recta (9).

Although the contribution of CO_2 trapping (if any) to urinary pCO_2 generation during hypercapnia is unknown, there is no a priori reason to believe that systemic blood pCO_2 would reflect vasa recta pCO_2 in hypercapnic subjects excreting an alkaline urine. Moreover, it is now known that in rats with acute respiratory acidosis, inner collecting duct pCO_2 is lower than that of the blood (38, 39). Based on these considerations, we reasoned that the use of the U−B pCO_2 achieved during bicarbonate loading could be a poor marker of collecting duct H^+ secretion under conditions where systemic blood pCO_2 is altered. Accordingly, we examined urinary pCO_2 generation and U−B pCO_2 gradients in hypercapnic rats studied under conditions where the urine was acid and when it was alkalinized in the presence and in the absence of accelerated carbonic acid dehydration.

Methods

Adult Sprague-Dawley rats (250–380 g) were allowed free access to food (Purina Lab Chow, Ralston Purina Co., St. Louis, MO) and water until the morning of each experiment. Studies using conventional clearance techniques were performed in anesthetized rats. Anesthesia was accomplished by the intraperitoneal administration of Inactin (100 mg/kg body weight). Tracheostomy was performed and one carotid artery and jugular vein were cannulated. Rats were connected to a rodent respirator (model 680, Harvard Apparatus Co., Inc., S. Natick, MA) to sustain a blood pCO_2 at the desired level. At the start of the experiment, ^{125}I-iothalamate diluted in saline (0.75 μCi/ml) was infused by an infusion pump at a rate of 0.024 ml/min throughout the course of the experiment as a marker of glomerular filtration rate (GFR). An equilibration period of 60 min was allowed before any collection was started. Urine samples were collected via a suprapubic catheter and drained into preweighed glass vials containing mineral oil; urine volume was determined gravimetrically. Blood samples were collected from the carotid artery during the middle portion of each clearance period.

Timed urine collections of ~20–30 min duration were obtained in the following groups of rats: in group Ia, eight normocapnic rats were infused with sodium bicarbonate (0.9-M solution) at a rate of 6 ml/h. At least two clearance collections were obtained from each rat after a urine pH of 7.8 or higher was achieved. The first urine collection with a urine pH of 7.8 or higher was discarded to eliminate mixing of urine of different pH's. After completion of this protocol, acute hypercapnia was induced in the eight rats by making them inhale a mixture of 10% CO_2, balance air via tracheal intubation. After 2 h of stable hypercapnia (blood pCO_2 range, 80–100 mmHg), urine collections were obtained while sodium bicarbonate infusion was continued throughout the experiment to keep urinary pH above 7.8.

In group Ib, nine rats were studied during normocapnia in response to a sodium bicarbonate infusion as described above. After obtaining at least two control clearance collections at a urinary pH of 7.8 or higher, moderate hypercapnia was induced using a mixture of 5% CO_2, balance air. Blood pCO_2 was kept constant between 68 and 78 mmHg for 2 h. Thereafter, experimental urine collections were completed while sodium bicarbonate infusion was administered as in group Ia.

In group IIa, seven rats were studied before and after induction of severe hypercapnia (blood pCO_2, 80–100 mmHg). Urine collections were obtained during the first and the second hour of sustained respiratory acidosis to determine the effect of this acid-base alteration on urinary pCO_2 and renal acid excretion.

In group IIb, seven normocapnic rats excreting a urine of low bicarbonate concentration (<3 meq/liter) were studied during 2 h and served as controls for hypercapnic rats excreting an acid urine (group IIa).

In group IIIa, seven rats were studied during severe hypercapnia as in group IIa except that a bolus dose of 5 mg of carbonic anhydrase followed by a continuous infusion (5 mg/h) was administered before induction of severe hypercapnia. Thereafter, carbonic anhydrase was infused at a rate of 5 mg/h during 2 h of sustained hypercapnia to examine urinary pCO_2 generation in the face of accelerated carbonic acid dehydration.

In group IIIb, six normocapnic rats were studied during the infusion of carbonic anhydrase as in group IIIb and served as normocapnic controls.

After completion of the above protocols, groups IIa, IIb, IIIa, and IIIb were infused with sodium bicarbonate. In groups IIIa and IIIb, the infusion of carbonic anhydrase was continued throughout the experiment. At least three urine collections were completed during the infusion of sodium bicarbonate (0.9-M solution) at a rate of 6 ml/h while blood pCO_2 was held constant.

In group IV, eleven rats were studied during 2 h of acute respiratory acidosis as in group IIa. Thereafter, they were infused with sodium bicarbonate at a slower rate (3 ml/h) in an effort to prevent a rise in blood pH to the alkalemic range that occurred in all previous groups studied during the course of sodium bicarbonate infusion.

In group IVa, a subset of six rats of group IV were further studied during carbonic anhydrase infusion (5 mg/h for 2 h after an initial bolus dose of 5 mg). Three collections of ~30 min duration were obtained while the rate of sodium bicarbonate infusion was maintained at ~3 ml/h.

In groups Va and Vb, ten normocapnic rats served as controls for group IVa hypercapnic rats. After completion of three urine collections during the infusion of sodium bicarbonate, five of them were given carbonic anhydrase (group Va) and five were not (group Vb, time controls). The rate of sodium bicarbonate infusion was maintained at ~3 ml/h throughout the protocol.

Analytical methods. The urine and arterial blood samples for pH and pCO_2 were measured on a Radiometer system (Radiometer America, Inc., Westlake, OH) immediately after collection. Bicarbonate concentration was calculated from the pH and pCO_2 using the Henderson-Hasselbalch equation as previously described (40, 41). Sodium and potassium were measured by flame photometry. Titratable acidity was calculated from the amount of 0.1 NaOH used to titrate a 1-ml sample (0.1 ml urine plus 0.9 ml distilled H_2O) up to a pH of 7.4. Ammonium was measured by a formulin titrimetric method (42).

Nonbicarbonate buffer capacity was measured using an automatic titrator (Radiometer America, Inc., model TTT 60, ABU 12) as previously described by Stinebaugh et al. (20). Briefly, HCl was added in sufficient amounts as to lower the pH of each urine specimen to below 2.5. The specimens were agitated for 30 min to ensure carbon dioxide loss. Thereafter, a titration curve from 2.5 to 8.5 was obtained by adding NaOH (0.1 M) to each of the urine samples. The nonbicarbonate buffer capacity was calculated from the pH 7.0–8.5 portion of

the titration curve as well as from 3.0 to 8.5 pH U. Either calculation yielded similar results, owing to the linearity of the titration curve. Carbonic anhydrase B from bovine erythrocytes was obtained from Sigma Chemical Co. (St. Louis, MO). Carbonic anhydrase activity in the urine was measured by the method of Maren et al. (43). This technique measures carbonic anhydrase activity by the rate of carbon dioxide hydration. Statistical analyses were performed using the *t* test for paired and unpaired data when appropriate. Values are means±SE.

Results

Effect of an acute elevation of blood pCO_2 on urine pCO_2 in rats excreting a highly alkaline urine (Table I, Fig. 1)

Effect of severe hypercapnia (group Ia). The data of rats infused with sodium bicarbonate before induction of hypercapnia are presented in Table I. In these rats, urine pCO_2 after alkalinization of the urine was 75±1.6 mmHg and U−B pCO_2 was 31±2 mmHg. Induction of acute hypercapnia in the face of continuous sodium bicarbonate infusion resulted in a fall in blood pH and a rise in plasma bicarbonate. The increase in blood pCO_2 (from 42±0.8 to 87±0.8 mmHg) was associated with an increase in urine pCO_2 (from 75±1.6 to 103±4.2 mmHg, $P < 0.001$) and a decrease in U−B pCO_2 (from 31±2 to 16±4.2 mmHg, $P < 0.005$). The rise in urine pCO_2 was of a greater magnitude than the rise in urine bicarbonate concentration (from 163±5.2 to 196±12 meq/liter); consequently, urinary pH fell (from 7.93±0.009 to 7.87±0.02, $P < 0.01$).

Effect of moderate hypercapnia (group Ib). In these rats, blood pCO_2 was increased to a lesser degree in order to investigate whether the low U−B pCO_2 observed in rats with severe hypercapnia had been, at least in part, the consequence of their higher blood pCO_2 per se, rather than failure to generate a sufficiently large urinary pCO_2.

The increase in blood pCO_2 (from 40±0.6 to 73±0.06 mmHg) resulted in a fall in blood pH despite an increase in plasma bicarbonate secondary to continuous sodium bicarbonate infusion. Urinary pCO_2 increased from 79±2.8 to 103±5.2 mmHg, $P < 0.005$; that is, to a value virtually identical to that observed in rats with severe hypercapnia (group Ia). This increase in urine pCO_2 occurred in the face of a modest rise in urine bicarbonate concentration (from 145±4.5 to 199±11 meq/liter) which was also virtually identical to that observed in rats with severe hypercapnia (group Ia) (Fig. 1).

In contrast to rats with severe hypercapnia, however, the U−B pCO_2 did not decrease significantly in response to induction of hypercapnia of moderate severity (from 39±2.5 to 30±4.9 mmHg). Hence, the U−B pCO_2 of rats with moderate hypercapnia was significantly higher than that of rats with severe hypercapnia (30±4.9 and 16±4.2 mmHg, respectively; $P < 0.05$). This difference was likely the consequence of a blood pCO_2 lower in rats with moderate hypercapnia than in rats with severe hypercapnia (73±0.6 and 87±0.8 mmHg, $P < 0.001$) because urinary pCO_2 was virtually identical between both groups (Fig. 1). Blood pH, plasma sodium, plasma potassium, and urine flow were not significantly different between these two groups of hypercapnic animals. After induction of hypercapnia GFR fell to a comparable level in both groups. A fall in GFR was also observed in normocapnic time control rats (group Vb) that are presented in Table V (see below).

Acid excretion during acute respiratory acidosis (Table II)

Induction of severe hypercapnia (group IIa) resulted in a significant fall in blood pH, a fall in urinary pH, and an increase in net acid excretion which were observed during the first hour of acute hypercapnia. The increase in titratable acid excretion was associated with an increase in phosphate excretion. A significant increase in ammonium excretion and a further increase in titratable acid and net acid excretion were observed during the second hour of sustained hypercapnia. The increase in ammonium excretion observed during the second hour of sustained hypercapnia was associated with a urinary pH identical to that observed during the first hour of hypercapnia. As anticipated, plasma K and plasma phosphate increased in response to hypercapnia. GFR did not fall significantly after induction of acute respiratory acidosis.

Urinary pCO_2 during acute respiratory acidosis

The urinary pCO_2 of group IIa rats was 57±9.1 mmHg in the first hour and 58±6.3 mmHg during the second hour of sustained hypercapnia. These values of urinary pCO_2 were significantly lower than blood pCO_2 (57±9.1 vs. 84±2.2 mmHg, $P < 0.02$, and 58±6.3 vs. 86±1.7 mmHg, $P < 0.005$). Therefore, in hypercapnic rats the U−B pCO_2 difference was markedly negative throughout the 2 h of observation (Fig. 2). The urinary pCO_2 of control rats (group IIb) was not significantly different than that of the blood (37±3.2 and 41±1.3 mmHg, respectively).

The urinary pCO_2 observed in group IIa rats during the second hour of hypercapnia was compared to that of normocapnic control rats (group IIb) which were excreting an acid urine. The urinary pCO_2 of hypercapnic rats was higher than that of normocapnic control rats (58±6.3 and 37±3.2 mmHg, respectively, $P < 0.02$) with a comparably low urine bicarbonate concentration (1.1±0.3 and 1.6±0.3 meq/liter, respectively). Consequently, urinary pH was lower in hypercapnic rats than in control rats (5.78±0.06 and 6.17±0.07, respectively, $P < 0.005$). Hypercapnic rats had a urinary phosphate concentration higher than that of normocapnic controls (72±17 and 29±6.3 mmol/liter, respectively, $P < 0.05$).

Urinary pCO_2 during acute respiratory acidosis and carbonic anhydrase infusion

In rats with acute respiratory acidosis infused with carbonic anhydrase (group IIIa), urinary pCO_2 was 50±4.3 and 55±4.7 mmHg during the first and second hour of hypercapnia, respectively. These values of urinary pCO_2 were significantly lower than blood pCO_2 (50±4.3 vs. 80±1.5 mmHg, $P < 0.001$, and 55±4.7 vs. 86±2.2 mmHg, $P < 0.001$, respectively). The urinary pCO_2 of these rats was also higher than that of normocapnic controls (group IIIb) infused with carbonic anhydrase (55±4.7 and 30±3.6 mmHg, respectively, $P < 0.005$) (Table III). The urinary pCO_2 of hypercapnic rats infused with carbonic anhydrase (group IIIa) was not significantly different from that of hypercapnic rats not receiving this enzyme (group IIa) (55±4.7 and 58±6.3 mmHg, respectively). Hence, the urinary pCO_2 of hypercapnic rats excreting a urine of low bicarbonate content was lower than that of the blood regardless of whether carbonic anhydrase was given or not. This resulted in a markedly negative U−B pCO_2 difference in hypercapnic but not in normocapnic rats (Fig. 2).

Effect of alkalinization of the urine on urinary pCO_2 in rats infused with carbonic anhydrase (Table III)

In hypercapnic rats (group IIIa), sodium bicarbonate infusion resulted in a significant rise in urine pCO_2 (from 55±4.7 to 78±2.7 mmHg, $P < 0.005$). In normocapnic control rats

Table I. Effect of Hypercapnia on Urine pCO_2 in Rats Excreting an Alkaline Urine

			Blood			Plasma			Fractional excretion			Urine				U − B
	GFR	V	pH	pCO_2	HCO_3	K	Na	Cl	Na	Cl	K	PO_4	HCO_3	pH	pCO_2	pCO_2
	ml/m	*ml/m*		*mmHg*	*meq/liter*	*meq/liter*	*meq/liter*	*meq/liter*	%	%	%	*mmol/liter*	*meq/liter*		*mmHg*	*mmHg*
Severe hypercapnia (group Ia, $n = 8$)																
	4.1	0.28	7.67	42	49	2.8	161	99	10	4.0	41	8.8	163	7.93	75	31
$NaHCO_3$ loading	±0.3	±0.02	±0.01	±0.8	±1.2	±0.08	±2.1	±0.8	±0.7	±0.4	±4.3	±1.1	±5.2	±0.009	±1.6	±2.0
$P<$	0.001	NS	0.001	0.001	0.001	NS	0.001	NS	NS	NS	0.02	0.05	0.02	0.01	0.001	0.005
$NaHCO_3$ loading + hypercapnia	2.3	0.24	7.55	87	75	3.0	181	98	14	3.0	27	12	196	7.87	103	16
	±0.2	±0.05	±0.02	±0.8	±3.9	±0.11	±2.8	±1.1	±2.0	±0.9	±4.2	±1.5	±12	±0.02	±4.2	±4.2
Moderate hypercapnia (group Ib, $n = 9$)																
	3.3	0.39	7.68	40	46	2.7	162	106	20	9.2	49	4.2	145	7.85	79	39
$NaHCO_3$ loading	±0.3	±0.03	±0.01	±0.6	±1.9	±0.1	±1.3	±0.6	±2.3	±1.9	±5.3	±1.4	±4.5	±0.1	±2.8	±2.5
$P<$	0.01	0.02	0.001	0.001	0.001	NS	0.001	0.001	NS	NS	0.005	0.02	0.001	NS	0.001	NS
$NaHCO_3$ loading + hypercapnia	2.1	0.26	7.58	73	68	2.7	185	103	20	5.9	28	6.0	199	7.85	103	30
	±0.2	±0.09	±0.01	±0.6	±1.9	±0.4	±3.4	±0.6	±1.3	±0.7	±2.7	±0.6	±11	±0.1	±5.2	±4.9
*	NS	NS	NS	<0.001	NS	NS	NS	<0.001	<0.025	<0.025	NS	<0.005	NS	NS	NS	<0.05

U − B pCO_2, urine minus blood pCO_2 difference. * Group Ia vs. group Ib during hypercapnia.

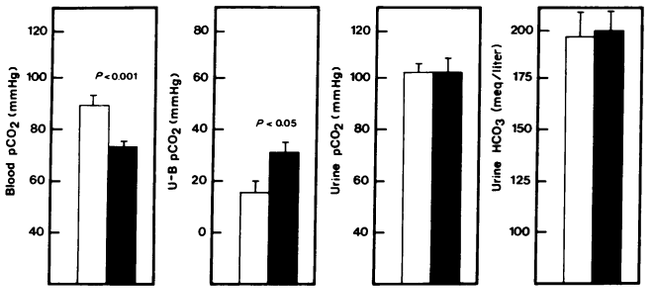

Figure 1. Blood pCO_2, U−B pCO_2, urine pCO_2, and urine bicarbonate concentration in rats with severe hypercapnia (open bars) and in rats with moderate hypercapnia (dark bars) infused with sodium bicarbonate. The lower U−B pCO_2 of rats with severe hypercapnia was totally accounted by their higher blood pCO_2 because urine pCO_2 was the same as that of rats with moderate hypercapnia.

(group IIIb), sodium bicarbonate infusion also resulted in a significant rise in urine pCO_2 (from 30.5±3.6 to 42±1.7 mmHg, $P < 0.02$). The increment in urinary pCO_2 elicited by sodium bicarbonate loading was higher in hypercapnic than in normocapnic rats (24±5.2 and 12±3.1 mmHg, respectively), but this difference did not achieve statistical significance ($P > 0.05$). During sodium bicarbonate loading, the urine pCO_2 of hypercapnic rats was significantly lower than that systemic blood pCO_2 (78±2.7 and 87±1.8 mmHg, respectively, $P < 0.02$), while that of normocapnic rats was not (42±1.7 and 40±0.3 mmHg, respectively). This resulted in a negative U−B pCO_2 in hypercapnic rats but not in controls (Fig. 2).

Urine pH was higher in normocapnic rats than in hypercapnic rats (8.08±0.05 and 7.90±0.02, respectively, $P < 0.005$) while urine bicarbonate concentration was not significantly different. GFR did not change significantly in either group while plasma sodium and plasma bicarbonate increased in both groups.

Effect of alkalinization of the urine on urinary pCO_2 in rats not infused with carbonic anhydrase (Table IV)

In the set of experiments presented in Table I, hypercapnia was induced in normocapnic rats that were excreting an alkaline urine and, therefore, had a high urine pCO_2. Thus, the additional rise in urinary pCO_2 that occurred in these rats could have been the result of abruptly raising the blood pCO_2 rather than the consequence of alkalinizing the urine. In the following set of experiments, sodium bicarbonate was infused into hypercapnic rats that were excreting a urine with low bicarbonate content. In hypercapnic rats (group IIa) in which sodium bicarbonate infusion resulted in a high blood pH, urinary pCO_2 rose from 58±6.3 to 98±4.9 mmHg ($P < 0.005$) in the absence of any significant change in blood pCO_2. This rise in urinary pCO_2 was therefore virtually identical to the rise in U−B pCO_2 (from −28±4.7 to 15±4.0 mmHg). The urinary pCO_2 achieved under these conditions was comparable to that of rats with moderate (group Ia) and severe hypercapnia (group Ib) in which blood pCO_2 was increased after alkalinization of the urine (compare Table IV with Table I). Blood pH after bicarbonate loading was also not significantly different (7.55±0.02, 7.58±0.01, and 7.53±0.02 in groups Ia, Ib, and IIa, respectively). Plasma sodium was higher in the hypercapnic rats presented in Table I (groups Ia and Ib) than in those presented in Table IV (group IIa). GFR fell significantly in groups Ia and Ib but not in group IIa, probably because the duration of the experiment was longer in groups Ia and Ib.

In normocapnic control rats (group IIb), urinary pCO_2 increased from 37±3.2 to 79±2.6 mmHg. Hence, the increment in urinary pCO_2 elicited by bicarbonate infusion while blood pCO_2 was kept constant (thereafter to be referred to as ΔpCO_2) was almost identical in hypercapnic rats (group IIa) and normocapnic rats (40±7.2 and 42±4.6 mmHg, respectively). After bicarbonate loading, urinary pCO_2 was higher in hypercapnic rats than in controls (98±4.9 and 79±2.6 mmHg, $P < 0.001$), but the U−B pCO_2 was lower in hypercapnic rats than in controls (15±4.0 and 39±2.5 mmHg, $P < 0.001$).

In hypercapnic rats (group IV) in which bicarbonate infusion resulted in a normal blood pH (7.38±0.01), urinary pCO_2 increased from 55±6.9 to 125±3.6 mmHg, $P < 0.001$. Thus, the ΔpCO_2 of these rats (70±5.6 mmHg) was greater than that observed in normocapnic rats (42±4.6 mmHg) (Fig. 3, *left*). It was also greater ($P < 0.005$) than that of hypercapnic rats (40±7.2 mmHg) with a higher blood pH (group IIa). The

Table II. Effect of Acute Respiratory Acidosis (Group IIa) on Acid Excretion

	GFR	V	Blood pH	Blood pCO_2	Blood HCO_3	Plasma K	Plasma PO_4	$UPO_4 \times V$	Urine pH	TA	NH_4	Net acid
	ml/min	*ml/min*		*mmHg*	*meq/liter*	*meq/liter*	*mmol/liter*	*μmol/min*		*μmol/min*	*μmol/min*	*μmol/min*
Prehypercapnia ($n = 7$)	3.4 ±0.4	0.01 ±0.03	7.42 ±0.01	40 ±1.5	25 ±1.5	3.5 ±0.2	2.0 ±0.1	0.1 ±0.05	6.5 ±0.2	0.2 ±0.05	0.4 ±0.1	0.5 ±0.2
P value	NS	NS	0.001	0.001	NS	0.01	NS	0.01	0.05	0.005	NS	0.005
Hypercapnia (1st h)	2.6 ±0.3	0.02 ±0.02	7.13 ±0.02	84 ±2.2	28 ±1.3	4.4 ±0.3	2.7 ±0.1	1.2 ±0.3	5.7 ±0.09	1.3 ±0.2	0.4 ±0.05	1.7 ±0.2
P value	NS	0.05	NS	NS	NS	NS	NS	0.01	NS	0.005	0.05	0.005
Hypercapnia (2nd h)	3.0 ±0.2	0.04 ±0.07	7.14 ±0.02	86 ±1.7	29 ±1.2	4.4 ±0.1	2.7 ±0.09	2.7 ±0.5	5.8 ±0.06	2.5 ±0.1	1.0 ±0.2	3.4 ±0.3
*	NS	0.02	0.001	0.001	NS	0.01	0.01	0.005	0.025	0.001	0.05	0.001

* Statistical analysis (*P* value) between hypercapnia (2nd h) and prehypercapnia.

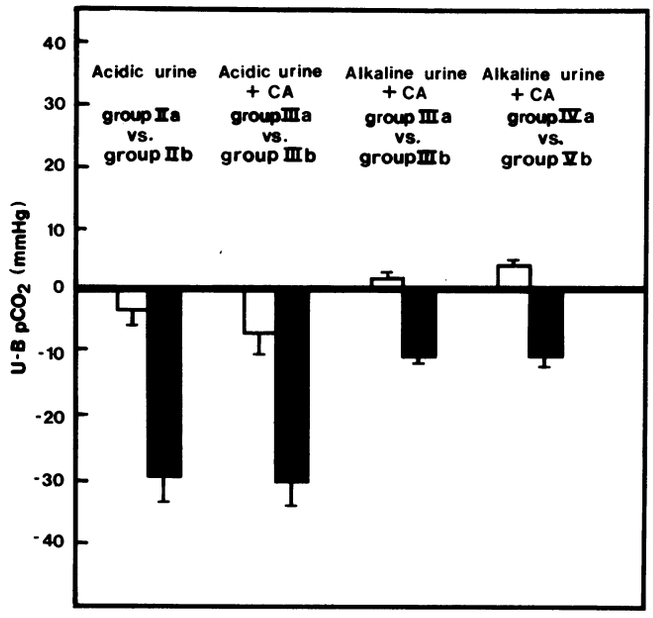

Figure 2. The U−B pCO_2 of hypercapnic rats (groups IIa, IIIa, and IVa) is compared with that of normocapnic control rats (groups IIb, IIIb, and Va). The U−B pCO_2 of hypercapnic rats was lower than that of normocapnic control rats when the urine was acid (with and without carbonic anhydrase infusion) and when it was alkalinized in the face of a continuous carbonic anhydrase (CA) infusion. □, control; ■, hypercapnia.

U−B pCO_2 of group IV hypercapnic rats (39±3.7 mmHg) was significantly higher than that of group IIa hypercapnic rats (15±4.0 mmHg) and not significantly different than that of normocapnic controls (group IIb) (39±2.5 mmHg). These differences in urine pCO_2 and U−B pCO_2 were observed in the face of a comparable increase in urine bicarbonate concentration in the three groups studied (Fig. 4). Plasma sodium also increased to a comparable level in the three groups. Plasma K was higher in hypercapnic rats than in controls.

Effect of carbonic anhydrase infusion on urine pCO_2 in rats excreting an alkaline urine (Table V)

In normocapnic control rats (group Va), carbonic anhydrase infusion resulted in a fall in urine pCO_2 (from 73±1.9 to 43±1.3 mmHg, $P < 0.001$) while urine bicarbonate concentration did not change significantly. Consequently, urine pH increased (from 7.87±0.03 to 8.04±0.04, $P < 0.02$). In hypercapnic rats (group IVa), the infusion of carbonic anhydrase also resulted in a fall in urine pCO_2 (from 122±5.7 to 77±2.2 mmHg, $P < 0.001$), while urine bicarbonate concentration did not change and urine pH increased (from 7.65±0.03 to 7.85±0.03, $P < 0.005$). The fall in urine pCO_2 elicited by carbonic anhydrase infusion was greater in hypercapnic rats than in normocapnic controls (−45±4.3 and −30±2.6 mmHg, respectively, $P < 0.02$) (Fig. 3, *right*).

Nonbicarbonate buffer capacity was not significantly different between normocapnic and hypercapnic rats either before (2.4±0.3 and 2.3±0.6 meq/liter per pH unit, respectively) or after carbonic anhydrase infusion (2.5±0.5 and 3.9±2.3 meq/liter per pH unit, respectively).

Carbonic anhydrase activity in the urine was not significantly different between hypercapnic and normocapnic rats (275±96 vs. 362±103 μg/ml, respectively). Likewise, the excretion of this enzyme was not significantly different between the two groups (31±8.3 vs. 23±4.0 μg/min, respectively). Carbonic anhydrase infusion was associated with a significant fall in GFR and urine flow in both hypercapnic and normocapnic rats. Neither parameter, however, was significantly different between hypercapnic and normocapnic rats.

In time control rats (group Vb) GFR also fell significantly, but urine, pCO_2, and U−B pCO_2 did not. The fall in GFR was likely the result of the prolonged duration of this experiment. Nonbicarbonate buffer capacity in the time control group was not significantly different than that of hypercapnic (group IVa) and normocapnic rats (group Va) infused with carbonic anhydrase.

After carbonic anhydrase infusion, the urine pCO_2 of hypercapnic rats (group IVa) fell to a level lower than that of the blood (77±2.2 and 86±0.9 mmHg, respectively, $P < 0.025$), while that of normocapnic rats (group Va) did not (43±1.3 and 40±1.0 mmHg, respectively). Thus, the relationship between urine pCO_2 and blood pCO_2 was virtually identical to that observed in hypercapnic (group IIIa) and normocapnic rats (group IIIb) in which the infusion of carbonic anhydrase antedated the infusion of bicarbonate (Fig. 2). As anticipated, the urinary pCO_2 achieved in both hypercapnic and normocapnic rats infused with sodium bicarbonate and carbonic anhydrase was markedly lower than that observed when this enzyme was not administered (compare Tables III and V with Table IV).

Discussion

This study demonstrates a clear dissociation between urine pCO_2 and blood pCO_2 during acute hypercapnia. In normocapnic rats excreting a highly alkaline urine, induction of severe hypercapnia resulted in a fall in the U−B pCO_2 gradient despite an increase in urine pCO_2 (group Ia, Table I). According to current views (9, 16–29, 36), the finding that the U−B pCO_2 achieved during bicarbonate loading is lower in hypercapnic rats than in normocapnic rats (Tables I and IV) requires the conclusion that collecting duct H^+ secretion is decreased by hypercapnia. In fact, such a conclusion was recently reached by another group of investigators who noted that the U−B pCO_2 gradient of hypercapnic dogs infused with sodium bicarbonate was lower than normal (23).

That the low U−B pCO_2 observed during bicarbonate loading reflects decreased collecting duct H^+ secretion during hypercapnia, however, is not likely correct. The urine pCO_2 of hypercapnic rats was higher than that of normocapnic rats, a finding that could be interpreted as indicative of enhanced collecting duct H^+ secretion in hypercapnic rats (i.e., the opposite interpretation of that portrayed by the U−B pCO_2 gradient). Alternatively, it could be argued that the higher urine pCO_2 observed in hypercapnic rats was purely the result of their higher blood pCO_2. The following findings, however, mitigate against the latter possibility.

First, rats with moderate hypercapnia (group Ib) exhibited a urinary pCO_2 virtually identical to that of rats with severe hypercapnia (group Ia) in the face of a comparable urine bicarbonate concentration (Fig. 1). This finding indicates that the U−B pCO_2 was influenced by changes in systemic blood pCO_2 rather than by changes in urinary pCO_2 generation. Second, urinary pCO_2 did not increase to the level prevailing in the blood in hypercapnic animals excreting a urine of low

Table III. Urinary pCO_2 in Rats Infused with Carbonic Anhydrase

			Blood			Plasma			Fractional excretion				Urine				
	GFR	V	pH	pCO_2	HCO_3	Na	K	Cl	Na	Cl	K	HCO_3	pH	HCO_3	pCO_2	U − B pCO_2	Δ pCO_2
	ml/min	*ml/min*		*mmHg*	*meq/liter*	*meq/liter*	*meq/liter*	*meq/liter*	%	%	%	%		*meq/liter*	*mmHg*	*mmHg*	*mmHg*
Hypercapnia (group IIIa, n = 7)																	
CA infusion	3.4 ±0.5	0.09 ±0.01	7.18 ±0.02	86 ±2.2	30 ±1.2	145 ±1.1	4.2 ±0.3	115 ±3.3	3.5 ±0.4	4.0 ±0.4	23 ±1.3	0.5 ±0.4	5.90 ±0.07	1.2 ±0.3	55 ±4.7	−30 ±5.2	—
$P<$	NS	0.001	0.001	NS	0.001	0.001	0.02	0.02	0.001	0.02	0.01	0.001	0.001	0.001	0.005	0.02	
CA infusion + $NaHCO_3$ loading	2.9 ±0.4	0.37 ±0.05	7.51 ±0.02	87 ±1.8	70 ±4.4	163 ±1.1	3.6 ±0.09	106 ±0.5	20 ±1.7	13 ±2.5	33 ±1.7	32 ±3.5	7.90 ±0.02	161 ±6.1	78 ±2.7	−10 ±2.1	24 ±5.2
Controls (group IIIB, n = 6)																	
CA infusion	2.6 ±0.5	0.06 ±0.01	7.46 ±0.01	37 ±0.9	26 ±0.8	141 ±1.6	4.0 ±0.6	112.3 ±11.7	3.7 ±0.7	5.9 ±1.2	34 ±5.3	0.12 ±0.04	5.99 ±0.19	1.3 ±0.4	30 ±3.6	−6.7 ±3.0	
$P<$	NS	0.001	0.001	0.02	0.001	0.001	NS	NS	0.001	NS	NS	0.001	0.001	0.001	0.02	0.0025	
CA infusion + $NaHCO_3$ loading	1.8 ±0.3	0.19 ±0.1	7.73 ±0.02	40 ±0.3	53 ±2.6	160 ±1.8	3.0 ±0.1	110.5 ±5.1	20 ±2.4	12 ±3.0	35 ±2.5	32 ±1.9	8.08 ±0.05	137 ±18	42 ±1.7	1.8 ±1.8	12 ±3.1
*	NS	NS	0.001	0.001	0.025	NS	NS	NS	NS	NS	NS	NS	NS	NS	0.005	0.005	—
‡	NS	NS	0.001	0.001	0.01	NS	0.001	NS	NS	NS	NS	NS	0.005	NS	0.001	0.005	NS

ΔpCO_2, denotes the rise in urine pCO_2 elicited by sodium bicarbonate infusion. * Hypercapnic rats vs. normocapnic rats studied before $NaHCO_3$ loading. ‡ Hypercapnic vs. normocapnic control rats studied after $NaHCO_3$ loading.

Table IV. Effect of Alkalinization of the Urine on Urinary pCO_2 Generation at a Constant Blood pCO_2

			Blood			Plasma			Fractional excretion				Urine					
	GFR	V	pH	pCO_2	HCO_3	Na	K	Cl	Na	Cl	K	HCO_3	PO_4	pH	HCO_3	pCO_2	U − B pCO_2	Δ pCO_2
	ml/min	*ml/min*		*mmHg*	*meq/liter*	*meq/liter*	*meq/liter*	*meq/liter*	%	%	%	%	*mmol/liter*		*meq/liter*	*mmHg*	*mmHg*	*mmHg*
Controls (groups IIb, n = 7)																		
Base line	2.6 ±0.4	0.01 ±0.003	7.38 ±0.008	41 ±1.3	24 ±0.6	147 ±2.1	3.4 ±0.4	115 ±1.1	0.4 ±0.1	0.8 ±0.2	18 ±2.9	0.04 ±0.01	29 ±6.3	6.17 ±0.07	1.6 ±0.3	37 ±3.2	−3.4 ±3.6	—
$P<$	NS	0.001	0.001	NS	0.001	0.001	0.02	0.001	0.001	0.001	0.005	0.001	0.01	0.001	0.001	0.001	0.001	
$NaHCO_3$ loading	3.0 ±0.1	0.39 ±0.03	7.69 ±0.01	41 ±0.4	49 ±1.8	163 ±1.0	2.6 ±0.2	107 ±0.7	21 ±2.9	10 ±2.4	50 ±5.2	44 ±5.6	4.2 ±0.14	7.86 ±0.01	149 ±3.7	79 ±2.6	39 ±2.5	42 ±4.6
Hypercapnia, (group IIa, n = 7)																		
Base line	3.0 ±0.2	0.04 ±0.007	7.14 ±0.02	86 ±1.7	29 ±1.2	143 ±0.7	4.4 ±0.1	121 ±1.0	2.2 ±0.3	2.9 ±0.3	24 ±2.4	0.3 ±0.2	72 ±17	5.78 ±0.06	1.1 ±0.3	58 ±6.3	−28 ±4.7	—
$P<$	NS	0.001	0.001	NS	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.01	0.001	0.001	0.005	0.001	
$NaCHO_3$ loading	3.3 ±0.3	0.49 ±0.05	7.53 ±0.02	83 ±1.3	69 ±2.7	163 ±1.7	3.0 ±0.1	109 ±0.8	23 ±2.2	12 ±1.1	43 ±1.9	39 ±4.7	5.8 ±0.9	7.82 ±0.02	171 ±11	98 ±4.9	15 ±4.0	40 ±7.2
Hypercapnia (group IV, n = 11)																		
Base line	1.8 ±0.1	0.02 ±0.004	7.09 ±0.008	86 ±0.7	25 ±0.5	147 ±0.5	4.0 ±0.1	113 ±0.9	1.6 ±0.3	2.2 ±0.4	24 ±3.0	0.07 ±0.02	74 ±9.7	5.89 ±0.07	1.4 ±0.4	55 ±6.9	−31 ±6.9	—
$P<$	0.05	0.001	0.001	NS	0.001	0.001	0.025	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	
$NaHCO_3$ loading	2.4 ±0.2	0.25 ±0.03	7.38 ±0.01	86 ±0.8	49 ±1.3	165 ±1.6	3.4 ±0.2	105 ±1.2	18 ±1.4	8.8 ±1.3	43 ±2.4	35 ±2.3	12.4 ±1.2	7.69 ±0.02	165 ±10	125 ±3.6	39 ±3.7	70 ±5.6
*	NS	NS	0.001	0.001	0.001	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	0.005	0.001	NS
‡	0.05	0.01	0.001	0.001	NS	NS	0.02	NS	NS	NS	NS	NS	0.001	NS	NS	0.001	NS	0.005
§	0.02	0.001	0.001	NS	0.001	NS	NS	0.05	NS	NS	NS	NS	0.005	0.001	NS	0.001	0.001	0.005

pCO_2, rise in urine pCO_2 elicited by sodium bicarbonate infusion. * Hypercapnia (group IIa) vs. controls (group IIb) during $NaHCO_3$ loading. ‡ Hypercapnia (group IV) vs. controls (group IIb) during $NaHCO_3$ loading. § Hypercapnia (group IIa) vs. hypercapnia (group IV) during bicarbonate loading.

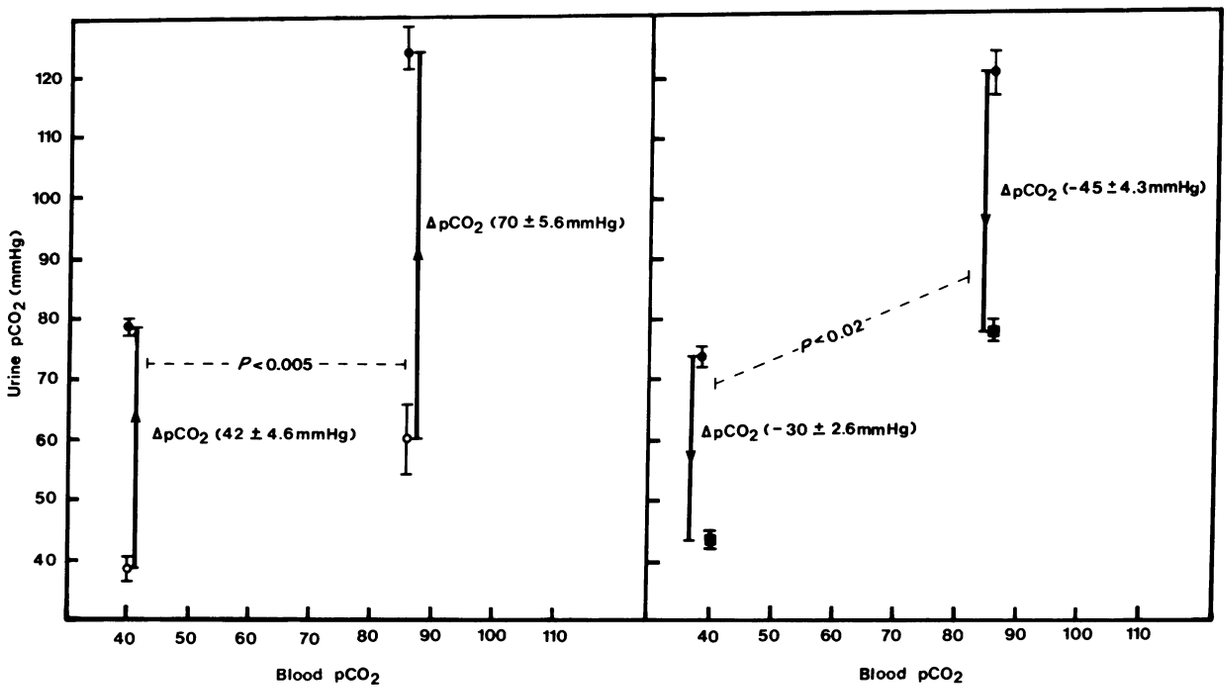

Figure 3. The relationship of urine pCO_2 to blood pCO_2 during normocapnia and hypercapnia. The *left* panel depicts the increase in urine pCO_2 elicited by sodium bicarbonate loading (i.e., the ΔpCO_2) in normocapnic rats (group IIb) and hypercapnic rats (group IV). Open circles denote urine pCO_2 when the urine was acid; closed circles denote the urine pCO_2 achieved during bicarbonate loading. The ΔpCO_2 was significantly higher in hypercapnic rats than in normocapnic controls ($P < 0.005$). The *right* panel depicts the fall in urine pCO_2 ($-\Delta pCO_2$) caused by carbonic anhydrase infusion to normocapnic rats (group Va) and hypercapnic rats (group IVa). Closed circles denote urine pCO_2 in an alkaline urine. Closed boxes denote that urine pCO_2 was measured during carbonic anhydrase infusion. The fall in urine pCO_2 elicited by carbonic anhydrase infusion was significantly higher in hypercapnic rats than in normocapnic controls ($P < 0.02$).

bicarbonate content; therefore, their U−B pCO_2 gradient was markedly negative (Fig. 2). This finding is in agreement with recent studies in dogs with acute hypercapnia (32) and in rats with chronic hypercapnia (33). Third, in hypercapnic rats excreting a urine of low bicarbonate content, the U−B pCO_2 was also negative when carbonic anhydrase was given to ensure complete carbonic acid dehydration and achievement of diffusion equilibrium between tubular and peritubular CO_2 (Table III, Fig. 2). Thus, even in the face of accelerated carbonic acid dehydration, urinary pCO_2 and blood pCO_2 did not equilibrate during hypercapnia. This finding differs from the study of Adrogue et al. (23) in dogs with severe hypercapnia. These authors reported that during carbonic anhydrase infusion, urine pCO_2 before bicarbonate loading was not significantly different from arterial blood pCO_2 within 30 min of induction of hypercapnia (23). Accordingly, they proceeded to use the U−B pCO_2 achieved after bicarbonate loading as a marker of distal acidification (23). The reasons for the discrepancy between our results and those of Adrogue et al. (23) are not readily apparent except that their studies were performed in only four animals of a different species. Fourth, in hypercapnic rats infused with carbonic anhydrase and sodium bicarbonate, urinary pCO_2 was still significantly lower than blood pCO_2 (78±2.7 vs. 87±1.8 mmHg, $P < 0.02$), which resulted in a negative U−B pCO_2 (Table III, Fig. 2). This finding further suggests that systemic blood pCO_2 does not reflect intrarenal pCO_2 even in the face of accelerated carbonic acid dehydration and in the presence of large urinary bicarbonate concentrations. Thus, factors other than equilibration between systemic blood pCO_2 and renal pCO_2 determine, at least in part, the urine pCO_2 in hypercapnic rats (see below).

Having established that the urinary pCO_2 of hypercapnic rats is lower than systemic blood pCO_2 we sought to determine the effect of alkalinization of the urine on urinary pCO_2 generation when blood pCO_2 was kept constant. We reasoned that if blood pCO_2 were not allowed to change the increment in urinary pCO_2 observed in response to sodium bicarbonate loading (i.e., the ΔpCO_2), it would provide an indirect qualitative measurement of collecting duct H^+ secretion. The ΔpCO_2 of hypercapnic rats with a high blood pH (group IIa) was comparable to that of normocapnic rats with a high blood pH (group IIb) (Table IV). The ΔpCO_2 of hypercapnic rats with a normal blood pH (group IV) was higher than that of normocapnic rats (Fig. 3, *left*), and than that of hypercapnic rats with a high blood pH (group IIa) (Table IV). The U−B pCO_2 achieved by hypercapnic rats with a high blood pH (group IIa), however, was again lower than that of normocapnic rats (group IIb) (15±4.0 and 39±1.5 mmHg, respectively), while that of hypercapnic rats with a normal blood pH (group IV) was the same (39±3.7 and 39±2.5 mmHg, respectively).

Urinary bicarbonate concentration, a major determinant of urinary pCO_2 generation (13–30), increased to a comparable level in both groups of hypercapnic rats and in normocapnic controls (Fig. 4). Since blood pCO_2 was not allowed to change,

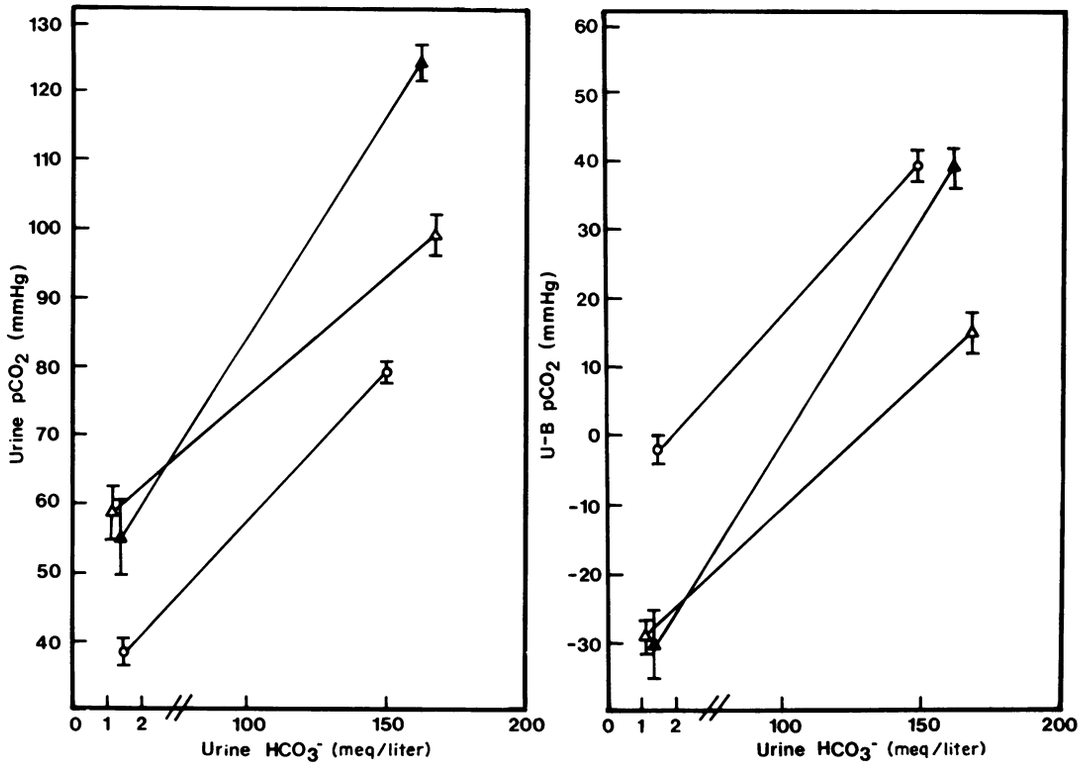

Figure 4. Urine pCO_2 as a function of urine bicarbonate concentration (*left*) and U−B pCO_2 as a function of urine bicarbonate concentration (*right*). At a comparably high urine bicarbonate concentration, rats with hypercapnia and a normal blood pH (group IV) (closed triangles) achieved a urine pCO_2 significantly higher than that of hypercapnic rats (group IIa) (open triangles) and normocapnic rats (group IIb) (open circles) that were alkalemic. The U−B pCO_2 of group IIa rats was lower, while that of group IV was equal to that of normocapnic control rats (group IIb) with a comparably high urine bicarbonate concentration.

the rise in urine pCO_2 was the consequence, at least in part, of collecting duct H^+ secretion into a bicarbonate-rich urine and not the consequence of equilibration between intrarenal and systemic blood pCO_2.

Urine pCO_2 and its derivatives as indices of distal acidification. The possible pitfalls of the urine pCO_2 and its derivatives as markers of collecting duct hydrogen ion secretion can be summarized as follows:

(a) U−B pCO_2 during bicarbonate loading. The use of the U−B pCO_2 observed in a highly alkaline urine as an index of collecting duct H^+ secretion requires that during bicarbonate loading, systemic blood pCO_2 be similar to that of medullary blood. DuBose (9), however, has recently demonstrated that in a highly alkaline urine the pCO_2 in the papillary collecting duct and adjacent vasa recta is markedly higher than that of the systemic blood (9). Thus, in normocapnic subjects infused with bicarbonate, intrarenal blood pCO_2 and systemic blood pCO_2 dissociate. Since the rise in urine pCO_2 in an alkaline urine occurs in the collecting duct and is, therefore, associated with a rise in vasa recta pCO_2, the use of the U−B pCO_2 serves no purpose because systemic blood pCO_2 is not a reflection of intrarenal blood pCO_2 (30). This fact, in our view, invalidates the use of the U−B pCO_2 gradient in a highly alkaline urine as an index of distal H^+ secretion even during normocapnia. This is not to say that the conclusions derived from numerous studies in normocapnic subjects using the U−B pCO_2 gradient as a marker of distal acidification need to be altered. When urinary pCO_2 and blood pCO_2 are about the same before bicarbonate loading (as is usually the case during normocapnia), the rise in urinary pCO_2 elicited by sodium bicarbonate infusion is equivalent to the U−B pCO_2 achieved under this condition (e.g., normocapnic rats, Table IV). Therefore, both parameters yield the same information in regards to the ability to generate CO_2 as a result of collecting duct H^+ secretion. When blood pCO_2 and urine pCO_2 are widely different, however, the U−B pCO_2 gradient achieved during bicarbonate loading becomes an artifact that gives no physiological information about collecting duct H^+ secretion. The artifactual nature of the U−B pCO_2 gradient is illustrated by our studies during hypercapnia, where it clearly underestimates distal H^+ secretion (Table IV, Fig. 4). This situation is the opposite of hypocapnia, where the U−B pCO_2 gradient can overestimate collecting duct H^+ secretion (44).

(b) Urine pCO_2 after bicarbonate loading. To take into account the relationship between urine pCO_2 and blood pCO_2 that prevailed before administration of sodium bicarbonate, we used the ΔpCO_2 as an index of collecting duct H^+ secretion (Table IV, Fig. 3, *left*). Blood pCO_2 was no longer a variable because it was not allowed to change, and the base-line urine pCO_2 (i.e., the pCO_2 of an acid urine) was known. Thus, the ΔpCO_2 is appropriate as a qualitative index of collecting duct H^+ secretion, in that it integrates the effects of both an acid and a highly alkaline urine on intrarenal CO_2 generation while it eliminates the blood pCO_2 as a variable. If the ΔpCO_2 were the best way to express the relationship between urinary pCO_2 generation and collecting duct H^+ secretion, one would have to conclude that collecting duct H^+ secretion was normal in

Table V. Effect of Carbonic Anhydrase Infusion on Urine pCO_2 in Rats Excreting an Alkaline Urine

	GFR	V	Blood			Plasma			Fractional ex		Urine				U − B pCO_2	−Δ pCO_2	Non-HCO_3 buffer capacity
			pH	pCO_2	HCO_3	Na	K	Cl	Na	Cl	PO_4	pH	HCO_3	pCO_2			
	ml/m	*ml/m*		*mmHg*	*meq/liter*	*meq/liter*	*meq/liter*	*meq/liter*	%	%	*mmol/liter*		*meq/liter*	*mmHg*	*mmHg*	*mmHg*	*meq/liter/ pH unit*
Controls (group Va, n = 5)																	
HCO_3 loading	2.3 ±0.2	0.14 ±0.02	7.62 ±0.02	38 ±1.5	37 ±0.6	156 ±1.3	3.2 ±0.04	108 ±1.7	11 ±1.7	5.6 ±1.2	13 ±2.9	7.87 ±0.03	146 ±7.6	73 ±1.9	35 ±2.6		2.4 ±0.3
$P<$	0.005	0.05	NS	NS	0.05	0.05	NS	NS	NS	NS	NS	0.02	NS	0.001	0.001		NS
HCO_3 loading + CA	1.1 ±0.3	0.08 ±0.01	7.64 ±0.01	40 ±1.0	43 ±1.7	162 ±2.0	3.7 ±0.5	108 ±2.2	13 ±2.4	6.7 ±2.0	11 ±1.7	8.04 ±0.04	128 ±12	43 ±1.3	3.1 ±1.9	−30 ±2.6	2.5 ±0.5
Hypercapnia (group IVa, n = 6)																	
HCO_3 loading	2.0 ±0.3	0.27 ±0.05	7.37 ±0.02	86 ±0.9	49 ±2.4	166 ±2.9	3.5 ±0.4	107 ±1.7	20 ±2.0	12 ±1.6	10 ±1.4	7.65 ±0.03	144 ±7.0	122 ±5.7	36 ±5.2		2.3 ±0.6
$P<$	0.02	0.02	NS	NS	NS	NS	NS	NS	NS	NS	NS	0.005	NS	0.001	0.001		NS
HCO_3 loading + CA	1.0 ±0.08	0.12 ±0.02	7.40 ±0.01	86 ±0.9	52 ±1.8	172 ±2.4	3.8 ±0.2	108 ±1.7	20 ±3.6	11 ±2.9	11 ±1.8	7.85 ±0.03	148 ±10	77 ±2.2	−9.0 ±2.8	−45 ±4.3	3.9 ±2.3
Time controls (group Vb, n = 5)																	
HCO_3 loading	2.4 ±0.6	0.18 ±0.02	7.63 ±0.02	40 ±0.9	41 ±1.4	154 ±2.0	3.5 ±0.1	111 ±3.4	15 ±2.4	9.7 ±2.2	8.5 ±2.0	7.81 ±0.02	135 ±16	75 ±5.4	35 ±5.1		1.9 ±0.4
$P<$	0.02	NS	0.005	NS	0.05	0.01	NS	NS	NS	NS	NS	NS	NS	NS	NS		NS
HCO_3 loading	1.5 ±0.4	0.13 ±0.03	7.67 ±0.02	41 ±1.3	47 ±3.4	165 ±2.3	3.8 ±0.3	110 ±1.9	17 ±4.5	104 ±3.8	9.4 ±1.5	7.85 ±0.04	138 ±18	70 ±3.8	29 ±3.4	−5 ±6.9	1.8 ±0.2
*	NS	NS	0.001	0.001	0.01	0.02	NS	NS	NS	NS	NS	0.005	NS	0.001	0.01	0.02	NS
‡	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	0.01	NS	0.001	0.001	0.01	NS
§	NS	NS	0.001	0.001	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	0.001	0.001	NS

* Hypercapnia (group IVa) vs. controls (group Va). (comparing data obtained during the second phase of the experiment). ‡ Time controls (group Vb) vs. controls (group Va) (comparing data obtained during the second phase of the experiment). § Time controls (group Vb) vs. hypercapnia (group IVa) (comparing data obtained during the second phase of the experiment).

hypercapnic rats that were alkalemic (group IIa) and enhanced in hypercapnic rats that were not alkalemic (group IV). This conclusion requires validation by measuring collecting duct pCO_2 and vasa recta pCO_2 before and after bicarbonate loading during sustained hypercapnia. While this information is currently not available, it is now known that in normocapnic rats, vasa recta pCO_2 and papillary collecting duct pCO_2 increase in a one to one ratio when the urine is highly alkaline (9). There is no a priori reason to believe that such a relationship does not exist during hypercapnia.

The ΔpCO_2 may be problematic, in that a number of variables (blood pH, plasma sodium, plasma potassium, volume status, medullary blood flow rate, etc.) change during bicarbonate diuresis as compared with base-line conditions. In this regard, the end point urine pCO_2 observed under identical conditions of bicarbonate diuresis, urine flow rate, and volume status is a more suitable marker of collecting duct H^+ secretion than the ΔpCO_2. If one were to use the end-point urine pCO_2, one would have to conclude that collecting duct H^+ secretion was enhanced in both groups of hypercapnic rats because the end-point urine pCO_2 was higher than that of normocapnic controls (Fig. 4). It is readily apparent, however, that the urine pCO_2 (i.e., that observed in a highly alkaline urine) fails to take into account the fact that the pCO_2 prevailing in the urine of hypercapnic animals before bicarbonate loading is higher than that of normocapnic rats. Thus, during hypercapnia, the end point urine pCO_2 observed after bicarbonate loading is uncertain as a marker of collecting duct H^+ secretion because it could overestimate it as opposed to the U−B pCO_2, which underestimates it.

We attempted to equalize urine pCO_2 and blood pCO_2 in hypercapnic rats by alkalinizing the urine in the presence of carbonic anhydrase infusion. We reasoned that the rise in urinary pCO_2 attributable to H^+ secretion into a bicarbonate-rich urine would be dissipated by accelerating carbonic acid dehydration, and that urinary pCO_2 and blood pCO_2 would be approximately the same. Urinary pCO_2 was about that of the blood in normocapnic rats but not in hypercapnic rats (Tables III and V, Fig. 2). The finding of a urinary pCO_2 lower than that of the blood in a highly alkaline urine rich in carbonic anhydrase deserves consideration. We think that accelerated consumption of CO_2 within collecting duct cells accounts for lowering of tubular CO_2 (and final urine CO_2) below systemic blood levels during hypercapnia. CO_2 is likely consumed and bicarbonate regenerated because of enhanced secretion of H^+, which under these conditions is titrated by bicarbonate and by nonbicarbonate buffer. The titration of H^+ by bicarbonate, in the presence of carbonic anhydrase, does not increase urine pCO_2 because of accelerated carbonic acid dehydration; the titration of H^+ by nonbicarbonate buffer consumes CO_2 in the process of bicarbonate regeneration and lowers urine CO_2. These two processes likely occur in both hypercapnic and normocapnic rats. That urine pCO_2 is lower than that prevailing in the blood in hypercapnic rats but not in normocapnic rats requires either enhanced collecting duct H^+ secretion or excess nonbicarbonate buffer in the hypercapnic animals. The urinary nonbicarbonate buffer capacity was comparable between hypercapnic and normocapnic rats (Table V), which suggests that enhanced collecting duct H^+ secretion was, at least in part, the cause of tubular CO_2 removal during hypercapnia.

The basic principle in using the urinary pCO_2 in a highly alkaline urine as a marker of distal acidification is to estimate the amount of pCO_2 generated from H^+ secretion and titration of bicarbonate buffer in the face of delayed carbonic acid dehydration (i.e., in the absence of carbonic anhydrase in the lumen of the distal nephron). In this regard, the difference in urine pCO_2 obtained in a highly alkaline urine not containing carbonic anhydrase and in an equally alkaline urine containing carbonic anhydrase should provide the best possible estimate of pCO_2 generated from H^+ secretion and titration of bicarbonate buffer. To attack this issue, we gave carbonic anhydrase to hypercapnic and normocapnic rats that were excreting an alkaline urine (Table V). This maneuver resulted in a greater fall in urine pCO_2 (i.e., $-\Delta pCO_2$) in hypercapnic rats than in normocapnic rats (Fig. 3, *right*). This clearly demonstrates that the amount of pCO_2 generated in the face of delayed carbonic acid dehydration (i.e., before carbonic anhydrase infusion) was greater in these hypercapnic rats than in controls, a finding which denotes enhanced collecting duct H^+ secretion. Blood pH was normal in the hypercapnic rats and alkalemic in normocapnic rats. Since changes in serosal pH (21, 45, 46) are known to modulate H^+ secretion, it seems likely that the difference in urinary pCO_2 generation noted between hypercapnic and normocapnic rats was determined by the prevailing blood pH rather than blood pCO_2. This is further suggested by the finding of a higher ΔpCO_2 in hypercapnic rats with a normal blood pH (group IV) than in those with a high blood pH (group IIa), while blood pCO_2 was identical in both groups (Table IV).

(c) Urinary pCO_2 before bicarbonate loading. The finding that urinary pCO_2 is lower than systemic blood pCO_2 in hypercapnic rats excreting an acid urine (Fig. 2, Tables III and IV) also deserves some comment. In an acid urine, secreted H^+ must react with buffers other than bicarbonate because the amount of filtered bicarbonate reaching the collecting duct is very low. Carbonic acid formation is necessarily low, thereby limiting the amount of CO_2 generated from the dehydration of this acid. In keeping with this notion, carbonic anhydrase infusion to hypercapnic rats in which the urine contained little bicarbonate did not result in a urinary pCO_2 different from that of hypercapnic rats not infused with the enzyme. In other words, the enzyme had no effect on urinary pCO_2 because bicarbonate was virtually absent from the lumen of the collecting duct. Collecting duct H^+ secretion when luminal bicarbonate is negligible results in the titration of nonbicarbonate buffer, a process that causes consumption of CO_2 because CO_2 combines with hydroxyl anions in the cell to regenerate bicarbonate.

Note, however, that the mechanism(s) whereby urine pCO_2 is lower than that of the blood in animals excreting an acidic urine has never been definitively elucidated. Rector (47) considered several possibilities other than collecting duct H^+ secretion. Enhanced bicarbonate reabsorption by a nonhydrogen ion secretory mechanism could lower tubular CO_2 (31, 47). This is not likely, because the amount of bicarbonate reaching the collecting duct in our experiments was minimal, as judged by the extremely low urinary bicarbonate concentration obtained during acute respiratory acidosis.

Another factor that could lower urine pCO_2 is mixing of urine containing excess of nonbicarbonate buffer with a poorly buffered acid urine (3, 47). Assuming that the nonbicarbonate buffer in excess during acute respiratory acidosis is phosphate, carbonic acid present in acidic urine will be consumed in the following reaction: $H_2CO_3 + Na_2HPO_4 \rightarrow NaHCO_3 + NaH_2PO_4$. We cannot exclude a role for excess urinary

phosphate buffer in lowering urine pCO_2 below systemic blood pCO_2, because urine phosphate concentration was higher in rats with acute respiratory acidosis than in controls. It must be emphasized that the hypothetical role of admixture of heterogeneous urine as a mechanism of lowering tubular CO_2 tensions does not negate the role of enhanced H^+ secretion as the main mechanism responsible for this phenomenon.

It is now known that during acute respiratory acidosis, renal cortical pCO_2 exceeds systemic blood pCO_2 (36) while inner medullary collecting duct pCO_2 is lower than arterial blood pCO_2 (38, 39). Therefore, tubular CO_2 is lost in transit from the superficial distal tubule to the inner medullary collecting tubule. Bengele et al. (39) and Gougoux et al. (32) explained the findings of an inner medullary collecting duct pCO_2 lower than that of the blood by postulating a loss of luminal CO_2 by diffusional flux into the lining epithelium. We think that this loss of tubular CO_2 (and thereby final urine CO_2) requires that H^+ secretion be enhanced and cell CO_2 consumed in the process of bicarbonate regeneration. As H^+ is secreted into a bicarbonate-poor luminal fluid, ammonium and acid phosphate are preferentially formed, thereby resulting in cell CO_2 consumption. This results in the creation of a lumen-to-cell and peritubular blood-to-cell CO_2 gradient and tubular CO_2 removal.

The titration of secreted hydrogen ions by nonbicarbonate buffer must result in the formation of either ammonium or titratable acidity or both. That urinary pH fell while titratable acidity and ammonium excretion increased in response to hypercapnia (Table II) is also consistent with the notion that the low U−B pCO_2 of these rats was achieved in association with enhanced collecting duct H^+ secretion. The increase in ammonium excretion observed in the second hour of sustained hypercapnia was unassociated with a fall in urine pH (Table II), which suggests that ammonia production increased. This is in agreement with several studies showing that overall kidney acidification is increased during hypercapnia (48–56) and with recent work showing a prompt adaptive increase in ammoniagenesis in response to acute hypercapnia (57). While proximal nephron segments are the major site of ammonia production, it is now known that a large portion of ammonium and net acid excreted in the final urine are added to the medullary collecting during respiratory acidosis (39) as well as metabolic acidosis (58, 59).

Based on all the above considerations, we believe that there is no evidence for the notion that distal acidification is decreased during acute hypercapnia (23). In view of recent direct studies showing that hypercapnia does not increase acidification above normal in either the superficial distal tubule (60) or in the inner medullary collecting duct (39), it seems likely that during hypercapnia acidification is either normal or enhanced only in the outer portion of the medullary collecting duct and/or the cortical collecting duct.

In conclusion, this study shows that hypercapnic rats have a urinary pCO_2 lower than systemic blood pCO_2 when the urine is acid and when it is alkalinized in the face of accelerated carbonic acid dehydration. These findings suggest that during acute hypercapnia, CO_2 is removed from the collecting duct owing to consumption of cell CO_2 in the process of bicarbonate regeneration via collecting duct H^+ secretion and titration of nonbicarbonate buffer. During bicarbonate loading, rats with hypercapnia have a U−B pCO_2 lower than or equal to that of normocapnic control rats, but the rise in urine pCO_2 elicited by this maneuver when blood pCO_2 is kept constant is equal or higher in hypercapnic rats than in normocapnic rats, depending on the prevailing blood pH. In hypercapnic rats, the U−B pCO_2 underestimates collecting duct H^+ secretion because this parameter is artificially influenced by systemic blood pCO_2, a variable that does not reflect vasa recta pCO_2 during bicarbonate loading. The ΔpCO_2 is a more appropriate qualitative index of collecting duct H^+ secretion than the U−B pCO_2, because it is not influenced by systemic blood pCO_2 and takes into account the urine pCO_2 prevailing before bicarbonate loading. A more precise evaluation of collecting duct H^+ secretion requires the infusion of carbonic anhydrase to estimate the amount of pCO_2 generated from H^+ secretion and titration of bicarbonate buffer when carbonic acid dehydration is accelerated and when it is delayed.

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