The Effect of Hyperventilation on Distal Nephron Hydrogen Ion Secretion

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ABSTRACT This study was designed to determine the effect of acute hyperventilation on distal nephron hydrogen ion secretion. The blood P_{CO_2} declined and stabilized rapidly when bicarbonate loaded rats were hyperventilated. In contrast, the urine P_{CO_2} declined slowly, resulting in an early increase in the urine minus blood (U-B) P_{CO_2} which could not be obliterated by carbonic anhydrase infusion. Within approximataely 50 min, the U-B P_{CO_2} in the hyperventilated and carbonic anhydrase infused rats approached zero. Consequently, equilibrium between collecting duct urine and arterial blood P_{CO_2} was then presumed to exist. This provided the basis for the subsequent studies on a series of rats. The U-B P_{CO_2} decreased from a control of 22±1 mm Hg (mean±SEM) to 11±2 mm Hg (mean±SEM) with hypocapnia, and rose again to its control value when the blood P_{CO_2} returned to prehyperventilation values. This decline in U-B P_{CO_2} with acute hyperventilation could not be attributed to changes in urine flow, phosphate, or bicarbonate excretion, suggesting, therefore, a decrease in distal nephron (probably collecting duct) hydrogen ion secretion with acute hyperventilation. Possible pitfalls in the interpretation of the U-B P_{CO_2} are illustrated.

INTRODUCTION

The effect of hyperventilation on the rate of hydrogen ion secretion in the terminal segments of the nephron has not been elucidated. Stanbury and Thomson (1) suggested that the electrolyte excretion patterns observed during hypocapnia might be due to inhibition of hydrogen ion secretion in the kidney. Micropuncture studies have shown that hyperventilation decreases hydrogen ion secretion in the proximal and distal convoluted tubules (2), but information about the response of the collecting duct to this maneuver is not available. The collecting duct is probably the most important segment of the nephron for achieving the minimum urine pH and thereby for augmenting acid excretion. In addition, the collecting duct has a major regulatory role in homeostasis under a variety of conditions (3).

In the present study, we have attempted to characterize the effect of hyperventilation on the distal nephron. To accomplish this, we have estimated distal nephron hydrogen ion secretion by utilizing the urine minus blood (U-B)[1] P_{CO_2} gradient (4). The U-B P_{CO_2} decreased during hyperventilation. This result cannot be explained by changes in urinary volume, bicarbonate, or buffer excretion. We propose that hyperventilation results in a reduction in distal nephron hydrogen ion secretion, presumably in the collecting duct.

METHODS

Experimental protocol. Male Wistar rats (250–300 g), obtained from Woodlyn Farms, Guelph Ontario, were allowed free access to food (Purina Lab Chow) and water before the experiment. Sodium bicarbonate, (20 g/l) and glucose (50 g/l) were added to the drinking water 2 days before the experiment to facilitate alkalinization of the urine. Rats were anesthetized with inactin, 100 mg/kg intraperitoneally. Intravenous infusions were administered into the jugular vein. Blood pressure was monitored by a femoral artery catheter connected to a mercury manometer. Blood samples were collected from the femoral artery into heparinized capillary tubes. The urine for pH and P_{CO_2} was collected anaerobically into capillary tubes via a suprapubic catheter in the bladder. pH and P_{CO_2} determinations were performed immediately after the collection as previously described (5). Urine for flow rate and all other parameters was collected into pretared vessels.

After induction of anaesthesia, 0.3 M sodium bicarbonate was infused at a rate of 100 μl/min throughout the experiment. When the urine pH and P_{CO_2} remained constant for three consecutive periods, a steady state was assumed to exist and blood was collected for pH and P_{CO_2}. After the

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

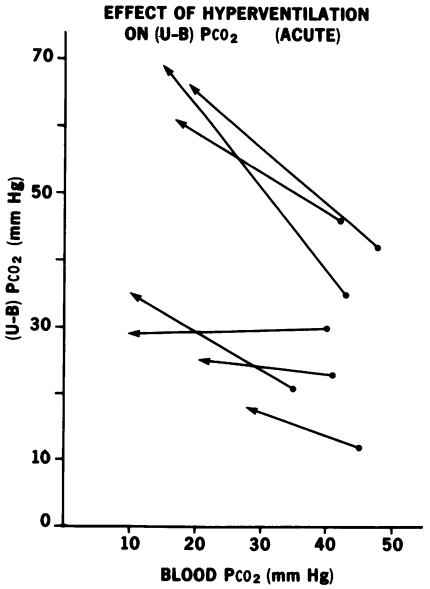

FIGURE 1 Each line represents values from a single rat. During control ventilation the U-B P_{CO_2} was 30±4.6 mm Hg (mean±SEM). With 5–15 min of acute hyperventilation, the U-B P_{CO_2} rose to 43.3±8.1 mm Hg (mean±SEM) giving an increased Δ U-B P_{CO_2} of 13.7±4.6 mm Hg, $P < 0.025$. ●—Before hyperventilation, ▲—Acute (5–15 min) hyperventilation.

control period, hyperventilation was induced with a mechanical respirator (Phipps and Bird small animal respirator, model no 7088-600, Phipps and Bird Inc., Richmond, Va.). In the experiments reported in Figs. 1–3, samples were obtained after the blood P_{CO_2} had declined to a steady-state value (blood P_{CO_2} in two consecutive samples stable within 3–5 mm Hg). In the experiments reported in Tables I and II, by contrast, samples were collected after the urine P_{CO_2} had remained constant for three consecutive periods. The animals were hyperventilated for approximately 90 min before the experimental specimens were taken. The respirator was then disconnected and the measurements were repeated after 15–30 min of spontaneous ventilation. If the blood P_{CO_2} remained low, the animal was placed in a covered box where the inspired gas contained humidified 5% CO_2; 95% O_2. Two rats hyperventilated spontaneously during the first period. In these animals steady-state values were obtained during hyperventilation and then normocapnia was induced by exposing them to the 5% CO_2; 95% O_2 gas mixture. Subsequently, they were removed from the box and allowed to resume spontaneous hyperventilation. Since the results were similar with both methods of hyperventilation, the data from both groups were combined.

Carbonic anhydrase was injected intravenously as a 2-mg bolus followed by a continuous infusion at a rate of 20 μg/min. Glomerular filtration rates (GFR) were measured with tritium labeled inulin using a 5-μCi priming dose followed by a sustaining dose of 6 μCi/h.

Analytical Methods. The blood and urine samples for pH and P_{CO_2} were measured on a Radiometer model PHM-72 digital acid base analyzer. Urine bicarbonate concentrations were calculated from the pH and P_{CO_2} using a pK as determined from the formula: $pK = 6.33 - 0.5\sqrt{\mu}$ (6) and a solubility coefficient of 0.0309. Sodium and potassium were measured by flame photometry as previously described (4). Phosphorus was measured by a modified method for the photoelectric colorimeter (7). Organic anions were measured by the titrimetric method of Chan (8). Tritium was measured on a Beckman LS 230 liquid scintillation counter (Beckman Instruments, Inc., Cedar Grove, N. J.) as previously described (5). Statistical analyses were performed by using a t test on paired observations.

Materials and supplies. All chemicals were of analytical grade. Tritium labeled inulin was obtained from New England Nuclear, Boston, Mass.; inactin from Henley and Co., Inc., New York; gas mixtures from Gas Dynamics, Toronto, Ontario; carbonic anhydrase from Sigma Chemical Co., St. Louis, Mo.; and reference buffers from Radiometer Co., Copenhagen, Denmark.

RESULTS

With acute hyperventilation, the blood P_{CO_2} decreased rapidly and stabilized while the urine P_{CO_2} declined more slowly. This resulted in an immediate rise in the U-B P_{CO_2} of 13.7±4.6 mm Hg, $P < 0.025$ (control U-B P_{CO_2} was 30±4.6 mm Hg, acute hyperventilation U-B P_{CO_2} was 43.3±8.1 mm Hg, mean±SEM, $n = 7$) (Fig. 1). To evaluate the possibility that the initial increase in the U-B P_{CO_2} was due to the failure of the arterial blood to reflect the medullary P_{CO_2}, or to mixing of urines with a higher and lower P_{CO_2}, carbonic anhydrase was infused into control and acutely hyperventilated rats. These results are shown in Fig. 2 and demonstrate that the U-B P_{CO_2} gradient was immediately abolished in the control rats (1±0.2 mm Hg) ($n = 4$) but not in the acutely hyperventilated rats. Therefore, the initial increase in the U-B P_{CO_2} could not be attributed to a distal nephron mechanism involving delayed dehydration

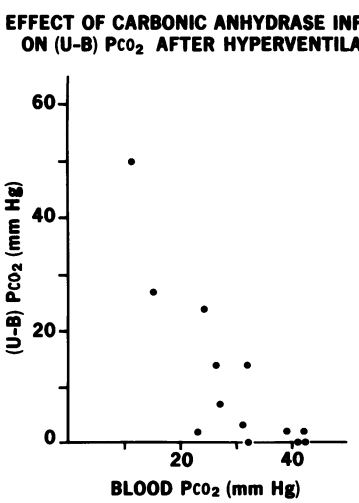

FIGURE 2 Each dot represents an individual rat. In the nonhyperventilated rats infused with carbonic anhydrase, the U-B P_{CO_2} gradient was not significantly different from zero (1±0.2) mm Hg (mean±SEM). In rats with a blood P_{CO_2} < 30 mm Hg and infused with carbonic anhydrase, the U-B P_{CO_2} ranged from 2 to 50 mm Hg when measured after 5–15 min of hyperventilation.

TABLE I

Effect of Hyperventilation on U-B P_{CO_2}

Rat	Normocapnia		Hypocapnia		Normocapnia		Hypocapnia	
	B P_{CO_2}	U-B P_{CO_2}	B P_{CO_2}	U-B P_{CO_2}	B P_{CO_2}	U-B P_{CO_2}	B P_{CO_2}	U-B P_{CO_2}
	mm Hg		*mm Hg*		*mm Hg*		*mm Hg*	
1	32	19	25	6	33	18		
2	32	20	19	9	30	18		
3	34	24	28	10	35	24		
4	40	21	27	4	39	22	26	9
5			29*	16	40	25	26	16
6			28*	17	36	24	28	17

B, blood.

* These animals were hyperventilating spontaneously and therefore the initial period was treated as a hypnocapnia one. Each value represents the mean of the values in three consecutive periods.

of carbonic acid, that is to an increase in distal nephron hydrogen ion secretion. In a separate group of rats, carbonic anhydrase was infused for 50 min after acute induction of hyperventilation. The U-B P_{CO_2} in hyperventilating rats was 32±4 mm Hg (mean±SEM, $n = 6$), 10 min after carbonic anhydrase infusion but decreased virtually to zero after approximately 50 min (2.5±1.0 mm Hg, $n = 6$) when equilibrium between arterial blood and renal medulla was assumed to exist. Therefore, the control U-B P_{CO_2} values were compared to values obtained after at least 50 min of hyperventilation in subsequent studies. As shown in Fig. 3, the U-B P_{CO_2} gradient decreased significantly ($P < 0.01$) as a result of steady-state hyperventilation. Furthermore, the results reported in Table I demonstrate that U-B P_{CO_2} gradient returned to control values when the blood P_{CO_2} returned to prehypocapnia values.

Other factors known to affect the U-B P_{CO_2} are the rate of buffer excretion, urine flow, and bicarbonate excretion. There was no significant fall in the urine phosphate excretion as a result of hyperventilation when the control and experimental periods were compared by the paired *t*-test (Table II). The analysis of paired data, however, reveal that there were significant decreases in both urinary flow rate and bicarbonate excretion rate during hyperventilation (Table II). GFR's were measured in three experiments and the results were similar in both states (2.20±0.5 vs. 2.01±0.6 ml/min in normal and hyperventilated rats, respectively). Calculated bicarbonate reabsorption rates in these experiments were 33.2 meq/l GFR in the control period and 27.5 meq/l GFR after 60 min of hyperventilation.

The U-B P_{CO_2} was decreased during hypocapnia when the results were compared at similar rates of urine flow, and bicarbonate, and phosphate excretion (Table III). For these data, a group of bicarbonate loaded rats with bicarbonate excretion rates between 1.5 and 8.0 μeq/min and phosphate excretion rates between 0.5 and 1.2 μmol/min was chosen for the normocapneic group for comparison with the hyperventilation data. (Methods for

TABLE II

Effect of Hyperventilation on the U-B P_{CO_2} and on Parameters Influencing U-B P_{CO_2}

	Summary of data										
	Blood			Urine							U-B
	pH	P_{CO_2}	H_{CO_3}	pH	P_{CO_2}	$[H_{CO_3}^-]$	$H_{CO_3}^-$	Phosphate	Organic anions	Flow	P_{CO_2}
		mm Hg	*meq/liter*		*mm Hg*	*meq/liter*	*μeq/min*	*μmol/min*	*μeq/min*	*μl/min*	*mm Hg*
Control	7.58	36	33	7.81	58	104.8	11.2	0.96	14.1	120	22
	±0.04	±1	±2.2	±0.06	±2	±19.6	±2.2	±0.23	±2.5	±31	±1
Hyperventilation	7.68*	26‡	30	7.84	37‡	71.9	4.0*	0.72	8.0*	72‡	11‡
	±0.02	±1	±1.3	±0.07	±3	±9.0	±0.5	±0.14	±1.5	±16	±2

These values represent the means and standard error of the means for control and hyperventilation data on animals from Table I.

* $P < 0.05$ for paired observations.

‡ $P < 0.01$.

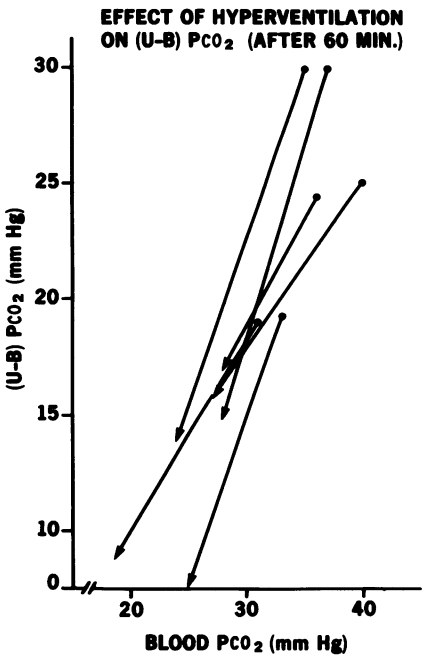

FIGURE 3 Each line represents values from a single rat. During control ventilation, the U-B P_{CO_2} was (22±1) mm Hg (mean±SEM), ($n = 7$). With steady state 60 min hyperventilation the U-B P_{CO_2} fell to (11±2) mm Hg (mean ±SEM).

preparation of animals, infusion protocols, and sample analyses were identical). The mean bicarbonate excretion rates were 4.7±0.6 and 4.0±0.5 μeq/min, urine flow rates were 52±10 and 72±16 μl/min and phosphate excretion rates were 0.80±0.08 and 0.72±0.14 μmol/min, respectively, in the normal and hyperventilating rats. The U-B P_{CO_2} was 26.2±1.5 mm Hg in the control group and was 11±2 mm Hg in the hypocapneic group.

DISCUSSION

The U-B P_{CO_2} gradient is a qualitative index of distal nephron hydrogen ion secretion. The physiologic basis for this assumption has been discussed previously (4). Briefly, carbonic acid is formed in the lumen of the distal nephron from the interaction of secreted hydrogen ions with the lumenal bicarbonate. Since carbonic anhydrase activity is virtually absent in this area of the nephron (9), the dehydration of carbonic acid is delayed until it reaches the lower urinary tract, where, because of unfavorable surface: volume relationships, little CO_2 is reabsorbed. It has been shown (10) that blood P_{CO_2} is a reasonable estimate of renal vasa recta P_{CO_2} in rats infused with sodium bicarbonate. Therefore, the U-B P_{CO_2} is an index of distal nephron net hydrogen ion secretion.

Several criteria must be met before one can use the U-B P_{CO_2} to assess distal nephron hydrogen ion secretion. The U-B P_{CO_2} must be measured during steady-state conditions. As shown in Fig. 1, the U-B P_{CO_2} gradient was increased after acute hyperventilation. This would indicate an increased distal nephron hydrogen ion secretion only if the blood P_{CO_2} accurately reflected renal medullary P_{CO_2}. In acute hypocapnia this may not be the case if there is inadequate time for equilibration. The U-B P_{CO_2} would overestimate distal nephron hydrogen ion secretion in this circumstance. The U-B P_{CO_2} could also overestimate distal hydrogen ion secretion if the collection contained urine with a higher P_{CO_2} formed before hyperventilation.

The elevation in the urine minus vasa recta P_{CO_2} due to distal nephron hydrogen ion secretion is abolished by carbonic anhydrase infusion (11). Carbonic anhydrase infused into acutely hyperventilated rats resulted in a fall in the U-B P_{CO_2} but not to levels which approximated the blood P_{CO_2} (Fig. 2). In contrast, the same dose of carbonic anhydrase caused the urine P_{CO_2} to virtually equal the blood P_{CO_2} in the normocapniac animals, confirming the results of others (9, 11, 12). The U-B P_{CO_2} approached zero after 50 min in acutely hyperventilated rats infused with carbonic anhydrase, indicating that a steady state had been achieved between arterial blood and collecting duct urine by this time. Detailed studies were then carried out to study the effects of hyperventilation on U-B P_{CO_2} after allowing 50 min equilibration time in order to achieve this steady state. There was a significant reduction in the U-B P_{CO_2} gradient during hypocapnia which returned to control values with cessation of hyperventilation (Table I).

Assuming steady-state conditions, the degree of elevation of the U-B P_{CO_2} is influenced by the interplay of several factors in addition to distal hydrogen ion secretion. These include rates of buffer excretion, urine

TABLE III

Effect of Hyperventilation on the U-B P_{CO_2} at Matched Rates of Urine Flow Phosphate and Bicarbonate Excretion

	Normocapnia	Hypocapnia
Blood P_{CO_2}, *mm Hg*	40.7±1.7	26±1*
Bicarbonate excretion, *μeq/min*	4.7±0.6	4.0±0.5
Phosphate excretion, *μmol/min*	0.80±0.08	0.72±0.14
Urine flow rate, *μl/min*	52±10	72±16
(U-B) P_{CO_2}, *mm Hg*	26.2±1.5	11±2*

The animals selected in the normocapnia group ($n = 16$) were those in which the urine flow rate phosphate excretion rate, and bicarbonate excretion rate were comparable to those observed in the hypocapnia group ($n = 6$) reported in Table II. The methods for preparation of animals, infusion protocols, and sample analyses were identical for both groups. Results are reported as mean±SEM.

* $P < 0.01$.

flow, and bicarbonate delivery. The major urine buffer is phosphate. There is a direct correlation between the U-B P_{CO_2} and the urine phosphate concentration (13). Phosphate infusion elevates the urine P_{CO_2}, presumably by delaying the dehydration of carbonic acid (14). There was no decline in urine phosphate concentration or excretion rates, however, during acute hyperventilation (Table II). Therefore, alterations in urine phosphate excretion are excluded as an important factor in our studies. Urine flow rate is another factor requiring consideration. Reid and Hills demonstrated that the urine P_{CO_2} varies inversely with urine flow (15). In these studies, both the U-B P_{CO_2} and urine flow rate fell with hyperventilation (Table I). Therefore, these results cannot be attributed to the changes in flow rate. Bicarbonate excretion rate can also influence the urine P_{CO_2} (16). Although the urine pH exceeded 7.55 in our studies, the urine bicarbonate excretion rates decreased with hyperventilation (Table II). We therefore examined the U-B P_{CO_2} at comparable rates of bicarbonate excretion in both normo- and hypocapnia (Table III). The U-B P_{CO_2} was 26.2±1.5 mm Hg during normocapnia when the bicarbonate excretion rate was 4.7±0.6 μeq/min whereas the U-B P_{CO_2} was significantly reduced to 11±2 mm Hg when the blood P_{CO_2} was 28±1 mm Hg and bicarbonate excretion 4.0±0.5 μeq/min. Urine flow rate and phosphate excretion rates were comparable between groups. Furthermore, examination of the data of Thompson and Barrett (17) demonstrates that when the bicarbonate excretion rate is acutely reduced by constriction of the aorta in bicarbonate loaded animals, the U-B P_{CO_2} did not decrease. Similar results were obtained in our laboratory when the bicarbonate excretion was decreased by acute unilateral ureteral constriction or by sodium nitroprusside infusion (unpublished observations). Thus, it appears unlikely that the hypocapnia-induced fall in U-B P_{CO_2} was caused by the reduced rate of bicarbonate excretion.

Another possible cause for the decreased U-B P_{CO_2} during hyperventilation is an increased rate of reabsorption of organic acids that were titrated in the distal nephron (18). This is unlikely in these experiments because the rate of organic anion excretion did not increase in bicarbonate-infused rats with superimposed hyperventilation.

The exact nephron segment responsible for the decreased U-B P_{CO_2} noted in our studies cannot be precisely determined. However, as the transit time from the distal tubule to renal pelvis is approximately 20 s, (19) and the H_2CO_3 dehydration time is quite rapid in urine of low buffer capability (20), we consider it unlikely that the U-B P_{CO_2} originates from areas other than the terminal collecting duct (18). It is conceivable that a reduction in the U-B P_{CO_2} during hyperventilation, rather than being due to decreased hydrogen ion secretion, might occur as the consequence of carbonic acid reabsorption in the collecting duct or to an increased rate of its dehydration within the lumen of this nephron segment. We feel that these latter possibilities are less likely and therefore interpret our data to indicate that the collecting duct responds to hypocapnia with a decrease in hydrogen ion secretion. If one assumes a semi-quantitative dimension for the U-B P_{CO_2}, the data presented would suggest that the largest proportional decrease in hydrogen ion secretion during hyperventilation occurs in the collecting duct. That is, the U-B P_{CO_2} decreased from 22±1 to 11±2, a 50% decline while the total bicarbonate reabsorption decreased only 17% from 33.2 to 27.5 mM/l GFR. This type of analysis, however, is potentially misleading in view of the lack of information regarding the quantitative aspect of the relationship between the collecting duct secretion and the U-B P_{CO_2}.

Previous investigators have provided indirect evidence that distal hydrogen ion secretion might be reduced in hypocapnia. Stanbury and Thomson (1) found an increased urine sodium and potassium excretion in respiratory alkalosis and suggested that this might reflect decreased distal nephron hydrogen ion secretion. Gennari et al. (21) demonstrated that net acid excretion was decreased in dogs with chronic hyperventilation. Malnic et al. (22) demonstrated in micropuncture studies that hypocapnia in rats reduced fractional and absolute bicarbonate reabsorption in both the proximal and distal tubules. They also note that hyperventilation reduced the dysequilibrium pH in the distal convoluted tubule of bicarbonate-infused rats. Their results strongly suggest a decrease in distal tubular hydrogen ion secretion.

Two major implications result from our studies. One is that the U-B P_{CO_2} provides a powerful tool to assess collecting duct hydrogen ion secretion in vivo provided the critical criteria are satisfied. In addition to controlling the excretion rates of phosphate, bicarbonate, and water, one must also control for the blood P_{CO_2} and ensure that measurements are made under steady state conditions. The other is that acute hyperventilation reduces collecting duct hydrogen ion secretion. This might be the basis for the reduced net acid excretion observed with more prolonged hypocapnia, leading to the greater fall in blood bicarbonate concentration associated with chronic hypocapnia (21, 23–25). In conclusion, we interpret our data to indicate that acute steady-state hyperventilation causes a decrease in distal nephron (presumably collecting duct) hydrogen ion secretion.

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