

Effect of Acute Hypercapnia on Renal and Proximal Tubular Total Carbon Dioxide Reabsorption in the Acetazolamide-treated Rat

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Abstract

The present study evaluates the effect of acute hypercapnia on renal total CO₂ (tCO₂) reabsorption after inhibition of renal carbonic anhydrase.

Simultaneous renal clearance studies and free-flow micropuncture studies of the superficial proximal tubule were performed on plasma-repleted Sprague-Dawley rats treated with acetazolamide, 50 mg/kg body weight. Acute hypercapnia (arterial PCO₂, 120 mmHg; blood pH, 7.02) was induced by ventilation with a 10% CO₂-90% O₂ gas mixture. Control rats (PCO₂, 49.5 mmHg, pH 7.34) were ventilated with room air. The renal fractional excretion of tCO₂ was ~20% lower in the hypercapnic group compared with the rats given acetazolamide alone. Acute hypercapnia reduced the fractional delivery of tCO₂ to the late proximal tubule by a comparable amount. The absolute proximal reabsorption of tCO₂ was increased by hypercapnia to 410±47 vs. 170±74 pmol·min⁻¹, *P* < 0.05. The single nephron glomerular filtration rate was 32.6±0.7 nl·min⁻¹ in the hypercapnic group and 43.8±1.7 nl·min⁻¹ in the rats given acetazolamide only, *P* < 0.01.

Acute hypercapnia enhances renal sympathetic nerve activity. To eliminate this effect, additional experiments were performed in which the experimental kidney was denervated before study. Denervation prevented the change in the single nephron filtration rate during acute hypercapnia, but absolute and fractional proximal tCO₂ reabsorption remained elevated in comparison to denervated controls.

The concentration of H₂CO₃ in the late proximal tubule, calculated from the measured luminal pH and bicarbonate concentration and the estimated cortical PCO₂, was higher in the hypercapnic group, which was a finding compatible with H₂CO₃ cycling from lumen into proximal tubular cell, which provided a source of hydrogen ions for secretion.

Introduction

Arterial blood CO₂ tension (PCO₂) is considered to be one of the major factors regulating the rate of tubular hydrogen ion (H⁺) secretion (1). Many clearance studies have documented that

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1. Abbreviations used in this paper: ACTZ, acetazolamide; AHC, acute hypercapnia; DNX, denervated; FE, fractional excretion; GFR, glomerular

filtration rate; SNGFR, single nephron glomerular filtration rate; tCO₂, total CO₂; TF, tubular fluid; TF/P, tubular fluid to plasma ratio; U/P, urine to plasma ratio.

acute hypercapnia (AHC)¹ enhances the maximal bicarbonate reabsorptive capacity of the kidney and decreases fractional bicarbonate excretion (2-5). It has also been shown that AHC can augment renal bicarbonate reabsorption in the presence of pharmacologic inhibitors of renal carbonic anhydrase such as acetazolamide (ACTZ). Thus, it has been suggested that carbonic anhydrase independent bicarbonate reabsorption is stimulated by hypercapnia (5-7).

Rector (8) has argued that, in the absence of carbonic anhydrase, the uncatalyzed rate of CO₂ hydration or hydroxylation cannot supply sufficient H⁺ to account for all of the bicarbonate reabsorbed under these circumstances. A number of possible means have been suggested as being responsible for maintenance of H⁺ secretion after inhibition of carbonic anhydrase. Of several possible mechanisms, recent studies have been interpreted to suggest that cycling of carbonic acid (H₂CO₃) from lumen into cell is a means by which H⁺ may be continuously made available for secretion in the proximal tubule of the ACTZ-treated rat under control circumstances and after the induction of metabolic acidosis or alkalosis (9).

In addition to a variety of systemic effects, AHC can reduce the glomerular filtration rate (GFR) and renal blood flow (10-14). The reduction in renal blood flow noted after AHC can be abolished by renal denervation, but not by maneuvers that block renin production (14). These findings suggest that the change in renal hemodynamics after AHC is related to increased renal sympathetic nerve activity. The findings of Anderson et al. (14) also suggest that the reduction in renal blood flow after AHC is proportionately greater than the change in the GFR. As a consequence, the filtration fraction and the peritubular capillary protein concentration are both increased. Recently, Cogan et al. (15) have demonstrated that proximal total CO₂ (tCO₂) reabsorption during inhibition of carbonic anhydrase may be modified by the peritubular protein concentration (15). Thus, any increase in proximal tCO₂ reabsorption after AHC in the ACTZ-treated rat may, at least in part, be an indirect result of the change in peritubular protein concentration.

In addition to the above observations, Shah et al. (16) and Chan (17) have shown that alpha adrenergic stimulation increases and denervation reduces proximal bicarbonate reabsorption. Thus, AHC may result in an increase in renal bicarbonate reabsorption via a direct effect of the AHC per se at the tubular level or as a consequence of stimulation of renal sympathetic nerve activity.

The present free flow micropuncture and clearance studies were undertaken to evaluate the effect of AHC on renal and proximal tubular tCO₂ reabsorption in the ACTZ-treated rat, and to examine the potential contribution of the increased sympathetic nervous activity induced by AHC on tCO₂ reabsorption under these conditions.

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ular filtration rate; SNGFR, single nephron glomerular filtration rate; tCO₂, total CO₂; TF, tubular fluid; TF/P, tubular fluid to plasma ratio; U/P, urine to plasma ratio.

Methods

25 Sprague-Dawley rats weighing 300–360 g were studied in four experimental groups. The rats were fed a standard rat chow diet and had free access to food and water before the experiments.

Anesthesia was induced by Inactin (100 mg/kg body weight, BYK Guldens, Konstanz, Federal Republic of Germany). The animals were placed on a thermostatically controlled heated table and body temperature was maintained between 37° and 38°C. Polyethylene catheters (PE 50) were inserted into the right femoral vein and artery for infusion of the various solutions and for blood pressure monitoring and sampling of arterial blood, respectively.

A tracheostomy was performed and the animals were connected to a Harvard rodent respirator. The rate and depth of ventilation were adjusted so as to keep arterial blood PCO₂ between 40 and 50 mmHg in the control animals ventilated with room air in the presence of ACTZ (groups A and D). AHC was induced in groups B and C by connecting the ventilator to a 10% CO₂, 90% O₂ gas mixture.

All animals were infused with homologous isoosmotic plasma obtained on the same day from another Sprague-Dawley rat. The plasma was infused from the start of the experiment at a rate of 20 µl/min. This amount of plasma resulted in a hematocrit at the conclusion of the study that was similar to that obtained before the abdominal and neck surgery. In addition, the rats received a noncolloid solution, either a modified Ringer solution (NaCl, 115 meq/liter; NaHCO₃, 25 meq/liter; KCl, 4 meq/liter) in normocapnic animals (groups A and D) or normal saline (0.9% NaCl) in the hypercapnic animals (groups B and C). These solutions were given at a rate of 25 µl/min simultaneously with the plasma infusion. Normal saline rather than Ringer's solution was administered to the hypercapnic animals to minimize the increase in plasma bicarbonate level induced by the hypercapnia, thus reducing the variability in filtered load among the experimental groups (15). An indwelling polyethylene catheter was inserted into the bladder for collection of the bladder urine in groups A and B. The clearance data reported for these groups represent the findings from both kidneys. In groups C and D, where denervation of the left kidney was performed, urine was collected separately from each kidney.

Micropuncture protocol. A ventral abdominal approach was used to expose the left kidney for micropuncture. Care was taken to minimize blood loss during the surgical preparation by clamping the bleeding points. The left kidney was separated from the surrounding tissue after ligation of the adrenal vessels. The kidney was placed in a lucite cup surrounded by oil-soaked cotton and bathed with mineral oil bubbled continuously with 5% CO₂ gas and maintained at 37° to 38°C. An arterial blood pressure of 100 mmHg or less at this point led to the exclusion of the animal from further study.

After completion of surgical preparation, a loading dose of 240 µCi ³H-methoxy inulin (New England Nuclear, Boston, MA) was given as a bolus followed by a sustaining infusion of the same amount per hour in the noncolloid solutions listed above. 30 min after the initiation of the inulin infusion, the modified Ringer solution was discontinued and ACTZ, 50 mg/kg body weight, was given as a bolus injection followed by a continuous infusion of the same amount per hour. ACTZ was dissolved in a 300 mM NaHCO₃ solution and was given at a rate of 25 µl/min in this solution in groups A and D to prevent reduction in plasma bicarbonate level due to ACTZ-induced urinary losses of bicarbonate. In the hypercapnic animals, groups B and C, ACTZ was dissolved in 50 µl of 300 mM NaHCO₃ solution and then mixed with 3 ml of saline solution and infused at a rate of 25 µl/min. Replacement of urinary losses of bicarbonate was not necessary in groups B and C.

30 min after ACTZ was begun, an arterial blood sample of 400 µl was taken for determination of pH and tCO₂, and a urine collection was begun into tared vials under mineral oil. A second blood sample was obtained at the end of the experiment. 4–6 late proximal convoluted tubules were identified by intratubular injection of 0.3% FD&C solution (Keystone Aniline & Chemical Co., Chicago, IL) with sharpened micropipettes (5–6 µm OD).

The following experimental groups were studied:

(a) *Group A (n = 7), ACTZ, and normal PCO₂.* This experimental group was ventilated with room air. Plasma PCO₂ was maintained between 40 and 50 mmHg by appropriate adjustment of the respirator. 4–6 timed tubular fluid collections were obtained from late proximal convoluted tubules by sharpened glass micropipettes (10–11 µm OD). Samples were immediately transferred to a chamber containing water-equilibrated mineral oil diffused with a 10% CO₂, 90% O₂ gas mixture and kept there until analysis. A simultaneous urine collection was obtained during the micropuncture period for clearance data.

(b) *Group B (n = 7) and ACTZ plus AHC.* This group was ventilated with 10% CO₂, 90% O₂ gas mixture during the experiment. Late proximal tubular samples and urine were obtained as in group A. The sample-containing pipettes were transferred to an oil chamber as in group A, with the exception that 15% CO₂ was in the chamber.

(c) *Group C (n = 6), ACTZ plus AHC and denervation.* This experimental group was treated identically to group B with the following exceptions: after separation of the left kidney from the surrounding tissue, a polyethylene catheter was inserted into the left ureter. Renal denervation was then performed according to the method described by Bello-Reuss and Gottschalk (18). The renal artery was exposed and the adventitia was stripped. The renal nerves were dissected and cut free. The renal artery was then wrapped with a narrow band of filter paper, previously soaked in a solution of 90% alcohol, 10% phenol, for 20–30 min. Care was taken to prevent contact between the phenol solution and the surface of the kidney during the procedure. Tubular samples were obtained as in the previous group. Urine was collected separately from the denervated (DNX) left kidney and the untouched right kidney.

(d) *Group D (n = 5), ACTZ, normal PCO₂, and denervation.* This group served as a control for group C to evaluate the effect of DNX per se on bicarbonate transport in the presence of ACTZ. The animals were ventilated as in group A with room air. Denervation was performed as described in group C. Tubular samples were obtained as previously described.

To evaluate the effectiveness of the denervation procedure, a separate group of eight rats was studied. Four rats underwent the denervation procedure as described for groups C and D, and four rats were sham operated. 72 h later the kidneys were removed and placed immediately in dry ice. Tissue norepinephrine content was subsequently determined (19). Three of the four denervated kidneys had undetectable levels of norepinephrine. The fourth contained 30 ng/g kidney. The norepinephrine levels in the sham-operated kidneys were 250, 365, 411, and 376 ng/g. We conclude that the denervation procedure used was successful.

In a separate series of studies, in rats prepared as in groups C and D, the intratubular pH was measured with a micro (7–9 µm OD) pH electrode. The electrode was manufactured as described by Puacacco and Carter (20) with the exception that the UO₂ content was reduced from 4 to 2%. No electrode was used that did not have a slope of at least 58 mV/pH unit when determined in vitro in a series of standard phosphate buffers (pH 4, 6.84, and 7.384) at 37°C. After its insertion into and removal from the tubule, the electrode was again tested in vitro. If the electrode potential deviated by >2 mV from the value obtained before tubular puncture, the data were discarded. The pH electrode was connected to a model 616 digital electrometer (input resistance 2 × 10¹⁴ Ω) (Keithley Instruments, Inc., Cleveland, OH) through an Ag/AgCl half cell (WPI, New Haven, CT). The electrometer was connected to a 7132A thermal recorder (Hewlett-Packard Co., San Diego, CA). This arrangement permitted us to read the potential of the electrode by the digital readout of the electrometer, yet graphically observe the characteristics and time course of the electrode response on the chart recorder. A calomel reference electrode, connected to the low input of the electrometer, and the cut end of the rat's tail were placed into a beaker containing Ringer's solution.

These micro pH electrodes can achieve values within 1 to 2 mV of end point in 20–30 s after placement in the pH buffers. When used in the tubular lumen, a stable reading, i.e., a variation of 1 mV or less, for 1 min, was recorded before the electrode was withdrawn. The pH mea-

measurements were made in late proximal sites located as described above. Two measurements were made in each of six rats.

Analysis. The volume of each tubular fluid sample was determined by measuring the length of the fluid column after transfer to a calibrated, constant bore quartz capillary. Great care was taken to maintain CO₂-equilibrated oil at both ends of the quartz capillary. The concentration of ³H-methoxy inulin in known volumes of tubular fluid samples, plasma, and urine was measured by scintillation counting using a LS7000 liquid scintillation counter (Beckman Instruments, Inc., Palo Alto, CA), as previously described from this laboratory (21). The tCO₂ concentration in tubular fluid samples was measured by microcalorimetry (22). The volume of samples used for this determination was 14.0–19.0 nl. Details of the general methodology used to handle the tubular fluid samples were reported previously (21).

Plasma and urinary tCO₂ were measured by a 960 carbon dioxide analyzer (Corning Medical and Scientific, Medfield, MA). Urine and arterial blood pH were measured with an Expandomatic Type IV pH meter (Beckman Instruments, Inc.). Plasma PCO₂ was calculated from tCO₂ and pH using pK_a of 6.1 and α of 0.0301. Plasma Na⁺ and K⁺ concentrations were measured by flame photometry. Plasma solids were measured by refractometry. The latter values were identical in all experimental groups. Therefore, no correction for plasma water was made in the calculation of TF/P ratio of inulin and tCO₂.

Calculations. Whole kidney GFR was calculated in the standard fashion. Single nephron glomerular filtration rate (SNGFR) = TF/P_{in} × tubular fluid flow rate, where TF/P_{in} is the tubular fluid to plasma inulin ratio. The SNGFR is expressed in nl · min⁻¹. Fraction of filtered tCO₂ delivered to end of proximal convoluted or to final urine = TF/P or U/P tCO₂/In × 100%, where TF/P or U/P tCO₂/In is the tubular fluid or urine to plasma tCO₂/inulin ratio. The absolute reabsorption of tCO₂ in the proximal convoluted tubule was calculated as the difference between the amount filtered and the amount delivered to the late proximal puncture site and is expressed in pmol · min⁻¹.

Statistics. One-way analysis of variance was used to evaluate statistical differences among the experimental groups. The Duncan test was used to evaluate the level of significance between each two groups that were compared (23). This test was applied only when the null hypothesis of no difference between the four experimental groups was rejected. The paired *t* test was used to compare clearance data between the control and experimental kidneys in the DNX animals (groups C and D). The data are expressed as mean ± SEM.

Results

A summary of the systemic and acid base data in the four groups is presented in Table I. A mild degree of respiratory acidosis (PCO₂ 49.5 and 50.8 mmHg) was observed in groups A and D, respectively. Increasing the tidal volume and/or rate of ventilation did not result in a further decrement in the arterial blood PCO₂. Similar results were obtained previously in the dog (5) and are presumably due to the inhibitory effect of ACTZ on erythrocyte carbonic anhydrase. Ventilation with a 10% CO₂, 90% O₂ mixture during carbonic anhydrase inhibition (groups B and C) resulted in a severe degree of hypercapnic acidosis of similar degree in both groups (Table I). Plasma tCO₂ was significantly elevated in the latter groups compared with their control counterparts (groups A and D). Calculated plasma bicarbonate was also significantly higher in the hypercapnic groups compared with the normocapnic animals. This increment in plasma bicarbonate occurred despite the fact that a saline solution was infused in the hypercapnic animals and reflects the nonbicarbonate buffer capacity of the plasma and extracellular fluid (24). The four groups were not statistically different with respect to their body weights, final blood hematocrit values, and arterial blood pressures. The latter finding is of interest since in the dog, hypercapnia may result in a significant fall in systemic blood pressure (25). Finally, plasma sodium concentrations were not statistically different in groups A and D from those in B and C (133.3 ± 1.19 meq/liter and 132.4 ± 0.94 meq/liter, respectively, *P* = NS). However, AHC (groups B and C) resulted in a significant increase in plasma potassium level (4.65 ± 0.09 meq/liter compared with 3.13 ± 0.05 meq/liter in groups A and D (*P* < 0.001)).

The clearance data from groups A and B are listed on Table II. AHC resulted in a prompt and significant reduction in urinary flow rate. Total GFR was not statistically different between the two groups. Urinary to plasma tCO₂ ratio was significantly decreased in the hypercapnic animals (ACTZ, 9.05 ± 0.16; ACTZ plus high PCO₂, 6.42 ± 0.12, *P* < 0.001). The fractional excretion

Table I. Systemic and Acid-base Data

Group	PCO ₂ mmHg	pH	Plasma tCO ₂ mM/liter	Plasma HCO ₃ ⁻ mM/liter	MBP mmHg	Body weight g	Hct %
A. ACTZ* (n = 7)	49.5 1.1	7.34 0.01	27.6 0.5	26.1 0.5	118 5	329 7	48.0 0.8
B. ACTZ + AHC (n = 7)	120.1 0.8	7.02 0.004	33.8 0.3	30.2 0.3	125 2	334 7	48.9 0.5
C. ACTZ + AHC + DNX (n = 6)	119.6 1.8	7.00 0.006	32.0 0.4	28.4 0.4	122 3	327 4	47.0 0.7
D. ACTZ + DNX (n = 5)	50.8 3.4	7.33 0.02	27.2 0.6	25.7 0.6	110 3	335 6	47.8 0.9
B vs. A	<i>P</i> < 0.01	<0.01	<0.01	<0.001	NS	NS	NS
C vs. B	<i>P</i> NS	NS	<0.05	<0.01	NS	NS	NS
C vs. A	<i>P</i> < 0.01	<0.01	<0.01	<0.01	NS	NS	NS
D vs. C	<i>P</i> < 0.01	<0.01	<0.01	<0.01	NS	NS	NS
D vs. A	<i>P</i> NS	NS	NS	NS	NS	NS	NS

MBP, mean arterial blood pressure; *n*, number of rats. Results on this and subsequent tables are expressed as the mean ± SEM.

Table II. Urinary Excretion Data

Group	V	GFR	U/P tCO ₂	Urine pH	FE tCO ₂
	$\mu\text{l} \cdot \text{min}^{-1}$	$\text{ml} \cdot \text{min}^{-1} \cdot 100 \text{ g body weight}^{-1}$			%
A. ACTZ (n = 7)	74.9 4.1	0.53 0.02	9.1 0.2	7.84 0.01	39.04 1.48
B. ACTZ + AHC (n = 7)	51.6 3.7	0.48 0.01	6.4 0.1	7.57 0.02	20.23 0.76
A vs. B	<i>P</i> < 0.005	NS	<0.001	<0.001	<0.001

V, urine flow rate; GFR represents results from both kidneys.

(FE) of tCO₂ was 39.04±1.48% in the normocapnic animals treated with ACTZ. This value is in general agreement with other published data regarding the effects of inhibition of renal carbonic anhydrase activity in the rat (26, 27), and demonstrates that ~60% of the filtered load of tCO₂ was reabsorbed. AHC resulted in a substantial reduction in fractional excretion (FE) tCO₂ to 20.23±0.76%, *P* < 0.001 (vs. group A), indicating that tCO₂ reabsorption in the presence of ACTZ was sensitive to changes in arterial blood CO₂ tension.

The micropuncture data from the superficial proximal convoluted tubule are presented in Table III. During administration of ACTZ, group A, the late proximal TF/P_{In} was 1.43±0.03 and the SNGFR 43.8±1.7 nl/min. The late proximal TF/P tCO₂ ratio was 1.19±0.08. Inhibition of renal carbonic anhydrase resulted in fractional delivery of tCO₂ of 85±6% to the end of proximal convoluted tubule, demonstrating that only 15% of filtered load of tCO₂ was reabsorbed in the proximal convoluted tubule under these circumstances. These data are similar to those obtained by others (15, 28) in plasma repleted rats.

Table III. Micropuncture Data

Group	TF/P _{In}	SNGFR	FLtCO ₂	TFtCO ₂	TF/P tCO ₂	ARtCO ₂	FDtCO ₂
		$\text{nl} \cdot \text{min}^{-1}$	$\text{pmol} \cdot \text{min}^{-1}$	mM/liter		$\text{pmol} \cdot \text{min}^{-1}$	%
A. ACTZ (n = 7)	1.43 0.03	43.8 1.7	1,189 43	33.3 2.8	1.19 0.08	170 74	85 6
B. ACTZ + AHC (n = 7)	1.70 0.08	32.6 0.7	1,085 28	35.3 2.1	1.05 0.06	410 47	62 4
C. ACTZ + AHC + DNX (n = 6)	1.47 0.06	47.7 2.0	1,527 71	26.22 1.6	0.83 0.05	653 102	58 5
D. ACTZ + DNX (n = 5)	1.38 0.05	44.7 2.8	1,210 109	29.7 1.7	1.08 0.05	262 45	78 3
B vs. A	<i>P</i> < 0.01	<0.01	NS	NS	NS	<0.05	<0.01
C vs. B	<i>P</i> < 0.01	<0.01	<0.01	<0.05	<0.05	<0.05	NS
C vs. A	<i>P</i> NS	NS	<0.01	<0.05	<0.05	<0.01	<0.01
D vs. C	<i>P</i> NS	NS	<0.01	NS	<0.05	<0.01	<0.05
D vs. A	<i>P</i> NS	NS	NS	NS	NS	NS	NS

FLtCO₂, single nephron filtered load of tCO₂; TFtCO₂, tubular fluid [tCO₂]; ARtCO₂, absolute reabsorption rate of tCO₂; FDtCO₂, fractional delivery of tCO₂ to end of proximal tubule.

Induction of AHC during carbonic anhydrase inhibition (group B) was associated with two major findings. First, under these conditions, the SNGFR was significantly decreased to 32.6±0.7 nl·min⁻¹, *P* < 0.01, and the TF/P_{In} was elevated to 1.70±0.08, *P* < 0.01, both compared with group A. Second, fractional tCO₂ reabsorption in the proximal tubule was markedly enhanced by AHC; i.e., fractional delivery of tCO₂ to the end of the proximal tubule was 62±4%, *P* < 0.01, compared with group A. The decrement in whole kidney tCO₂ excretion after AHC is therefore reflected by a corresponding change in fractional tCO₂ reabsorption in the superficial proximal tubule. The fact that the difference in the TF/P tCO₂ ratio between groups A and B did not reach statistical significance may be related to the simultaneous increment in proximal fluid reabsorption in group B after AHC.

The single nephron filtered load of tCO₂ in groups A and B were comparable (Table III). The absolute reabsorption of tCO₂ in group B, 410±47 pmol·min⁻¹, was significantly greater than that observed in group A, 170±74 pmol·min⁻¹, *P* < 0.05. To evaluate the contribution of renal sympathetic nerve activity to the renal response to hypercapnia, additional studies, groups C and D, were performed in which the left kidney was DNX before the study. Induction of AHC after denervation (group C) resulted in arterial blood pH and PCO₂ values similar to that noted in group B, Table I. There was a mild decrease in plasma tCO₂ as well as in the calculated plasma bicarbonate concentration in group C compared with group B.

Clearance data comparing the left DNX kidney with the right, untouched kidney in group C are presented in Table IV. Single kidney GFR was mildly elevated in the DNX kidney (0.28±0.01 ml/min compared with the innervated kidney, 0.24±0.01 ml/min, *P* < 0.01). This result is of interest in view of the finding that there was no significant difference in total kidney GFR between groups A and B. The reason for this discrepancy is unclear, but it may be that a change in whole kidney GFR, resulting from sympathetic overactivity after AHC, is rel-

Table IV. Comparison of Clearance Data from the Innervated and DNX Kidneys in Group C

	Innervated	DNX	<i>P</i>
GFR (ml · min ⁻¹ · 100 g body weight ⁻¹)	0.24±0.01	0.28±0.01	<0.01
FE tCO ₂ (%)	18.0±1.3	22.7±0.7	<0.02

actively small and can be depicted only when the DNX kidney is compared with the untouched kidney in the same hypercapnic animal. There was a slight but significant difference in FEtCO₂ between the two kidneys (innervated 18.0±1.3%; DNX, 22.7±0.7%, *P* < 0.02). Both values are similar to the value observed in group B. The fractional excretion of tCO₂ in the DNX kidney of group D, ACTZ alone, was 35.8±2.9%, which was significantly greater than the 22.7±0.7% noted in the DNX kidney in group C. Thus, renal sympathetic activity was not responsible for the enhanced renal tCO₂ reabsorption induced by the AHC.

Table III also lists the values obtained from micropuncture results from groups C and D, in which the left (experimental) kidney was DNX before study. DNX of the experimental kidney did not affect the response to ACTZ noted in the proximal tubule, group D vs. group A. The observation that DNX did not affect proximal tubular function in the absence of AHC (group D vs. group A) is of interest. These findings suggest that renal DNX may not affect the SNGFR and proximal tubular fluid reabsorption if baseline sympathetic nerve activity is not increased.

Renal DNX prevented a reduction in SNGFR and an increase in proximal TF/P_{in} ratio from occurring in response to AHC, group B vs. group C. Despite the DNX, an increase in proximal tCO₂ reabsorption was still observed in response to AHC, group C vs. group D. The TF/P tCO₂ ratio in group C, 0.83±0.05, was less than noted in group D, 1.08±0.05, *P* < 0.05. Fractional delivery of tCO₂ to the late proximal tubule after AHC, group C, was 20% less than in group D, which was approximately the same difference as was noted between groups A and B, the groups in which renal innervation was intact. The absolute proximal reabsorption of tCO₂ was significantly greater after AHC, group C, 653±102 pmol · min⁻¹, than in the denervated control, group D, 262±45, *P* < 0.01. The single nephron filtered load of tCO₂ was significantly greater in group C than in group D, Table III. However, after correcting the plasma tCO₂ concentration for the arterial PCO₂ in the two groups, the filtered load of bicarbonate was not different in the two groups, 1,352±52, group C, vs. 1,150±88 pmol · min⁻¹, group D, *P* = NS.

The measurement of tCO₂ in tubular fluid by microcalorimetry reflects not only measurement of bicarbonate in the sample, but that of dissolved CO₂ as well. Without precise knowledge of the renal cortical PCO₂, it is not possible to correct the measured tubular fluid tCO₂ for the latter value. In view of the difference between the systemic and renal cortical PCO₂ and an amplification of this difference during respiratory acidosis (28), correction of the tubular fluid tCO₂ concentration for the renal cortical PCO₂ will result in a greater change than correction of plasma tCO₂ for arterial blood PCO₂. Consequently, proximal TF/P bicarbonate ratios would be lower than the TF/P tCO₂

ratios shown with proportionately greater reductions in this ratio in AHC rats because of the difference in renal cortical PCO₂ as mentioned above. Given these considerations, it is clear that the rates of fractional and absolute tCO₂ reabsorption demonstrated above reflect corresponding changes in the reabsorptive rate of bicarbonate.

It is unlikely that the increase in single nephron filtered load of tCO₂ in group C, in contrast to group D, contributes significantly to the difference in absolute proximal absorption noted between the two groups. Variations in the filtered load of tCO₂ do not, of themselves, alter fractional proximal tCO₂ reabsorption in the ACTZ-treated rat (15). Accordingly, <20% of the difference in absolute tCO₂ reabsorption, in group C in comparison with group D, can be attributed to the higher filtered load present in the former group. Further, the differences in proximal tCO₂ reabsorption cannot be attributed to differences in tubular fluid flow rate (15) as they were identical in these two groups.

Intratubular pH measurements were performed in six late proximal convolutions of three normocapnic, ACTZ-treated, DNX animals, and in six tubules of three hypercapnic, DNX animals treated with ACTZ, Table V. Mean plasma pH and tCO₂ in the normocapnic group were 7.35±0.01 and 25.7±0.33 mM, respectively; they were 7.05±0.01 and 31.7±0.88 mM, respectively, in the hypercapnic group. These values are not statistically different from those obtained in experimental groups C and D, the groups that were handled identically to those in which the pH measurements were made. It is assumed, therefore, that the in situ pH measurements obtained in these animals accurately reflect the in situ pH in groups C and D in which determination of tCO₂ transport was made. The in situ late proximal intratubular pH was 6.90±0.01 in the animals treated with ACTZ only. This value is significantly different (*P* < 0.005) from the value of 6.58±0.02 obtained in the proximal segment of the animals treated with ACTZ and subjected to AHC.

Discussion

The findings of the present study confirm earlier observations (5–7) which demonstrate that renal bicarbonate reabsorption in the presence of an inhibitor of carbonic anhydrase may be significantly increased by elevation of the arterial PCO₂. An increase of ~20% in fractional tCO₂ reabsorption was noted after AHC in the present studies, indicating that carbonic anhydrase independent bicarbonate reabsorption was quite sensitive to changes in arterial PCO₂. Our data also demonstrate that the effect of AHC, in this experimental setting, is reflected by a cor-

Table V. Late Proximal Luminal pH Measurements

	ACTZ + AHC		ACTZ
Rat 1	6.62*		6.84
	6.55		6.84
Rat 2	6.51		6.95
	6.57		6.94
Rat 3	6.61		6.92
	6.65		6.91
Mean	6.58±0.02	<i>P</i> < 0.005	6.90±0.01

* Each measurement was obtained from a separate tubule.

responding change in $t\text{CO}_2$ reabsorption in the proximal convoluted tubule. This observation, however, does not exclude an effect of AHC in other nephron segments or nephron groups under these circumstances. Such a major impact of AHC on proximal bicarbonate reabsorption is of interest in view of the finding that in the normal rat, when renal carbonic anhydrase activity is intact, AHC induces only a mild effect on proximal bicarbonate reabsorption, or has no effect at all (29–32). Finally, our data show that AHC can have a marked influence on the SNGFR and fluid reabsorption of the superficial proximal tubule. These alterations appear to be mediated by renal sympathetic nerve stimulation, secondary to the AHC. However, the increase in $t\text{CO}_2$ reabsorption in the superficial proximal tubule after AHC was unrelated to indirect or direct effects of the increase in sympathetic activity, as renal denervation prevented AHC from affecting either the SNGFR and proximal fluid reabsorption; yet proximal $t\text{CO}_2$ reabsorption remained substantially elevated.

The increase in $t\text{CO}_2$ reabsorption in the innervated group B, in comparison with group A, may be attributable to several factors, and not only the result of the direct effect of an acute increase in PCO_2 on proximal H^+ transport. Cogan et al. (15) have suggested that proximal tubular $t\text{CO}_2$ reabsorption in the ACTZ-treated rat may vary directly with the peritubular capillary protein concentration. If AHC in the innervated group B increased the filtration fraction in superficial nephrons as a consequence of enhanced renal sympathetic nerve activity (14), such an increase could contribute to the increase in the $t\text{CO}_2$ reabsorption in this group. Second, renal sympathetic activity, of itself, can directly stimulate proximal $t\text{CO}_2$ reabsorption and could contribute to the increase noted in group B (16, 17).

In contrast to the findings in group B, the contribution of these indirect and direct effects of renal sympathetic activity would not be responsible for the increase in $t\text{CO}_2$ reabsorption noted after AHC in the DNX state. Denervation prevented a reduction in SNGFR after AHC, and we presume, based upon other studies (14), that no change in renal plasma flow occurred in these rats. Similarly, any direct effect of sympathetic nerve activity on $t\text{CO}_2$ reabsorption would be removed by the denervation. The results in the DNX studies, therefore, indicate that the effects of AHC on proximal $t\text{CO}_2$ reabsorption are not only mediated via stimulation of renal sympathetic activity.

After the administration of an inhibitor of renal carbonic anhydrase, fractional $t\text{CO}_2$ delivery to the end of the superficial proximal tubule remains remarkably constant when examined in the normal or plasma expanded rat or after the induction of metabolic acidosis or metabolic alkalosis (15, 33). Thus, as a consequence of the considerable variability of the filtered load of $t\text{CO}_2$ or some derivative thereof, noted in these diverse experimental settings, the constancy of fractional $t\text{CO}_2$ reabsorption is associated with large differences in absolute reabsorption. In contrast to the observations cited above, the present experiments indicate that AHC increases $t\text{CO}_2$ reabsorption in the proximal tubule of the ACTZ-treated rat independent of an increase in the filtered load.

Net bicarbonate transport in the proximal convoluted tubule is felt to be a function of the secretion of H^+ coupled with a small passive backleak of bicarbonate (34). In this segment, H^+ secretion is primarily a result of a Na^+/H^+ antiporter located in the luminal membrane (35). In the absence of an inhibitor of carbonic anhydrase, this segment demonstrates a remarkable capacity to reabsorb bicarbonate, particularly if the tubular flow

rate and the luminal bicarbonate concentration are optimal (36, 37). ACTZ treatment is felt to reduce proximal bicarbonate reabsorption by reducing the cellular supply of H^+ available for secretion, and, at least in part, by allowing luminal H_2CO_3 to accumulate, thereby inducing a pH gradient between cell and lumen unfavorable for proton secretion. The continued reabsorption of bicarbonate in the proximal tubule after ACTZ has been ascribed to several mechanisms (8, 38). Some of this bicarbonate can be attributed to the H^+ supplied by the uncatalyzed hydration and hydroxylation of CO_2 reactions, but the numbers so supplied are insufficient to account for the quantity of bicarbonate reabsorbed (27). Cogan et al. (27) have recently estimated the contribution of the uncatalyzed reactions to proximal bicarbonate reabsorption, taking into consideration the effects of alterations in intracellular bicarbonate concentration. Their estimates would predict that the uncatalyzed rate of H^+ secretion is insufficient to account for the rate of carbonic anhydrase independent reabsorption observed unless the intracellular pH were in excess of 8.5. Although it has been shown that the intracellular pH becomes more alkaline during carbonic anhydrase inhibition (39, 40), an intracellular pH of this magnitude, together with an intratubular acid disequilibrium pH, would result in a pH gradient across the apical membrane of ~ 2 pH units. The authors considered a H^+ gradient of this magnitude to be unlikely.

To determine if an increase in cell PCO_2 as a consequence of the AHC contributed significantly to the increase in proximal bicarbonate reabsorption noted in group C, in comparison to group D, we have extended the calculations of Cogan et al. (27) using slightly different kinetic constants (41). By computer modeling (see Appendix), we were able to estimate the rate of cellular H^+ production by the uncatalyzed reactions as a function of both intracellular pH and PCO_2 ranging from 40 to 180 mmHg, at a constant intracellular bicarbonate concentration of 25 mM. The absolute rate of $t\text{CO}_2$ reabsorption observed after AHC in group C is substantially higher than that predicted by the uncatalyzed reactions, even when the cortical PCO_2 is ~ 60 mmHg higher than the arterial PCO_2 . Thus, unless one assumes an extremely alkaline intracellular pH (pH of 9.0 at a cortical PCO_2 of 120 or 180 mmHg), the anticipated rate of the uncatalyzed reactions cannot account for the magnitude of the proximal bicarbonate reabsorption observed after AHC.

A number of years ago, Rector (8) proposed another mechanism that would account for a continuous supply of H^+ to a proton secretory process in the presence of carbonic anhydrase inhibition (8). He suggested that the H_2CO_3 , which accumulated in the lumen after ACTZ, might cycle from the lumen into the cell, where it would dissociate. Thus, H^+ would be available for secretion not dependent upon continuous production from the uncatalyzed hydration and hydroxylation of CO_2 . Cogan (9) has recently argued that the direct relationship between luminal bicarbonate concentration and bicarbonate reabsorption in the presence of ACTZ is the result of a proximal H^+ secretory mechanism that is limited by the lumen to cell pH gradient. In his view, H^+ secretion will vary directly with the luminal bicarbonate concentration and, at a constant luminal pH, the luminal H_2CO_3 concentration will parallel the luminal bicarbonate concentration. The proportional change in H_2CO_3 concentration with luminal bicarbonate concentration will allow more H_2CO_3 to enter the cell at high luminal bicarbonate concentrations, thus accounting for more bicarbonate to be reabsorbed than when the luminal bicarbonate concentration is lowered.

The increase in proximal $t\text{CO}_2$ reabsorption in the present

studies cannot be attributed precisely to the same mechanism. Based upon approximations of the renal cortical PCO_2 (below), luminal bicarbonate concentrations in the AHC groups would be approximately equal to (group B) or below (group C) their appropriate controls, groups A and D, respectively. Further, in contrast to the hypothesis put forward by Cogan (9) to explain parallel changes in luminal H_2CO_3 and bicarbonate concentrations at a constant luminal pH, our data indicate that, at least under our experimental circumstances, the luminal pH is not fixed. Yet, our data are compatible with luminal H_2CO_3 as a source for H^+ for secretion and with an increase in luminal H_2CO_3 after AHC. The luminal pH in the rats treated as in group D was 6.90. Assuming a cortical PCO_2 of 60 mmHg (33), and using a luminal bicarbonate concentration of 27.9 mM and a pK_a of 3.57, the calculated luminal H_2CO_3 concentration at this pH was 13 μM . No experimental data are available to indicate the cortical PCO_2 in the hypercapnic animals in group C, but it likely can be expected to exceed 150 mmHg (33). If we assume a cortical PCO_2 of 180 mmHg, a luminal H_2CO_3 concentration of 20 μM can be calculated from the luminal pH (6.58) and corrected luminal bicarbonate concentration.² The permeability of the apical membrane of the proximal convoluted tubule to H_2CO_3 and the H_2CO_3 concentration gradient across this membrane are unknown. Nevertheless, the higher luminal H_2CO_3 in the AHC rats is consistent with a greater potential source for H^+ production derived from H_2CO_3 , which moves from lumen to cell. Our studies, unfortunately, do not permit a precise determination of the amount of H_2CO_3 that has traversed the luminal membranes into the cell and contributed to H^+ secretion. Theoretically, this could be all of the H_2CO_3 produced by H^+ secretion that did not noncatalytically break down in the lumen. Luminal pH measurements and calculated H_2CO_3 concentrations are not reported for rats studied as groups A and B. The direct and indirect consequences of renal sympathetic stimulation after AHC (group B) prevents examination of the singular effects of the increase in PCO_2 per se on tCO_2 reabsorption, and likely these parameters as well.

If we assume that H_2CO_3 cycling may provide a continuous source of H^+ for secretion in the ACTZ-treated state, the ability of AHC to increase H^+ secretion relative to the control state requires explanation. The increase in proximal tCO_2 reabsorption after AHC presumably reflects stimulation of the Na^+/H^+ antiporter in the luminal membrane, or an altered luminal membrane permeability to H^+ . The activity of this antiporter is dictated, in part, by the pH gradient across the luminal membrane. In addition, Aronson et al. (42) have demonstrated in brush border membrane vesicles that a reduction in intravesicular pH stimulates the activity of the Na^+/H^+ antiporter in a manner that suggests allosteric interaction of H^+ with an activator or modifier site. Consequently, a parallel increase in the intracellular and intratubular concentrations of H^+ may potentially enhance Na^+/H^+ exchange without a measurable change in pH gradient across the luminal membrane. On the other hand, if AHC were to disproportionately increase intracellular H^+ concentration in the proximal tubule of the ACTZ-treated rat relative to the intratubular H^+ concentration, a more favorable pH gradient would be established across the luminal membrane and an increase in antiporter activity and tCO_2 reabsorption might be

2. The H_2CO_3 concentrations were calculated from late proximal values. Whether the same concentrations would exist along the entire length of the proximal tubule will require additional study.

anticipated. If a more favorable pH gradient were generated between cell and lumen by AHC, this may not be reflected by blood to lumen pH gradients. The pH gradients between blood and lumen were virtually identical in groups C and D.

In summary, the present studies indicate that AHC can dramatically increase renal tCO_2 reabsorption in the absence of renal carbonic anhydrase activity. An increase in tCO_2 reabsorption under these circumstances can be shown to occur in the proximal convoluted tubule. It is suggested that H_2CO_3 , cycling from luminal to cell, provides a continuous source of H^+ for secretion.

Appendix

The source of intracellular proton generation during carbonic anhydrase inhibition is the uncatalyzed reaction which consists of the following: (1) CO_2 hydration: $\text{CO}_2 + \text{H}_2\text{O} \xrightleftharpoons[k_{-1}]{k_1} \text{H}_2\text{CO}_3 \leftrightarrow \text{H}^+ + \text{HCO}_3^-$; and (2) CO_2 hydroxylation: $\text{H}_2\text{O} \rightleftharpoons \text{OH}^- + \text{H}^+ \quad \text{CO}_2 + \text{OH}^- \xrightleftharpoons[k_{-2}]{k_2} \text{HCO}_3^-$.

The latter reaction, which makes a significant contribution to the disappearance of CO_2 in aqueous solutions at $\text{pH} > 7.8$ (41) is an additional source of intracellular H^+ supply; at $\text{pH} > 10$, it dominates the uncatalyzed reaction. The relative contribution of the hydration and hydroxylation of CO_2 will be, therefore, pH dependent. The maximal rate of H^+ ion supply by the uncatalyzed reaction in the proximal nephron is:

$$\begin{aligned} (d\text{H}^+)/(\text{dt}) = & ([\text{CO}_2]k_1 - [\text{H}_2\text{CO}_3]k_{-1} \\ & + [\text{OH}^-][\text{CO}_2]k_2 - [\text{HCO}_3^-]k_{-2} \times V_e, \end{aligned}$$

where V_e = the volume of proximal tubule cells and $[\text{CO}_2]$, $[\text{H}_2\text{CO}_3]$, $[\text{OH}^-]$, and $[\text{HCO}_3^-]$ refer to the intracellular concentrations of these species.

Cogan et al. (27) have shown that the rate of the uncatalyzed H^+ ion supply $(d\text{H}^+)/(\text{dt})$ depends mainly on intracellular pH and bicarbonate concentration. Their calculation is based on the assumption that the intracellular carbonic acid concentration is in equilibrium with intracellular $[\text{H}^+]_i$ and bicarbonate $[\text{HCO}_3^-]_i$. Thus: $[\text{H}_2\text{CO}_3]_i = ([\text{H}^+]_i \times [\text{HCO}_3^-]_i)/(2.7 \times 10^{-4} \text{ M})$. Furthermore, the intracellular PCO_2 is constant and is similar to the cortical PCO_2 occurring during hypercapnia. To determine the influence of variations in PCO_2 on the H^+ generation by the uncatalyzed reaction, we extended their calculations to include cortical PCO_2 as an additional variable. Using a computer model we were able to predict the effect of alterations in cortical PCO_2 ranging from 40 to 180 mmHg on H^+ ion supply independent of changes in intracellular pH and bicarbonate concentration. The model uses the same tubular geometry data and kinetics as used by Cogan et al. (27), with the exception that k_1 and k_{-1} were replaced as follows: $k_1 = 4.8 \text{ min}^{-1}$; $k_{-1} = 1.92 \times 10^3 \text{ min}^{-1}$. These constants were considered by Maren (42) to be more accurate at 37°C. Fig. 1 shows a three-dimensional model relating the rate of H^+ ion production to both intracellular pH and PCO_2 at an $[\text{HCO}_3^-]_i$ of 25 mM. It is clear that the H^+ ion production by the uncatalyzed reaction increases as a function of intracellular pH and PCO_2 . A comparison of H^+ secretory rates at intracellular $[\text{HCO}_3^-]_i$ of 15 and 30 mM rather than 25 mM indicated that intracellular $[\text{HCO}_3^-]_i$ starts to be an important factor, albeit quantitatively minor, only when the intracellular pH is low, i.e., < 7.00 .

We now examine the predictions of the model in relation to the data obtained in our study during AHC, group C. In group C, arterial PCO_2 was 120 mmHg and plasma tCO_2 was 32.0 mM. Calculated plasma bicarbonate concentration is therefore 28.4 mM. The single nephron filtered load of bicarbonate was 1,355 $\text{pmol} \cdot \text{min}^{-1}$. To calculate absolute proximal bicarbonate reabsorption from proximal tCO_2 concentration of 26.2 mM, it is necessary to obtain the exact tubular PCO_2 that was not measured in our study. However, we can take two extreme possibilities: (1) tubular PCO_2 is higher by 50% than arterial PCO_2 , i.e., ~ 180

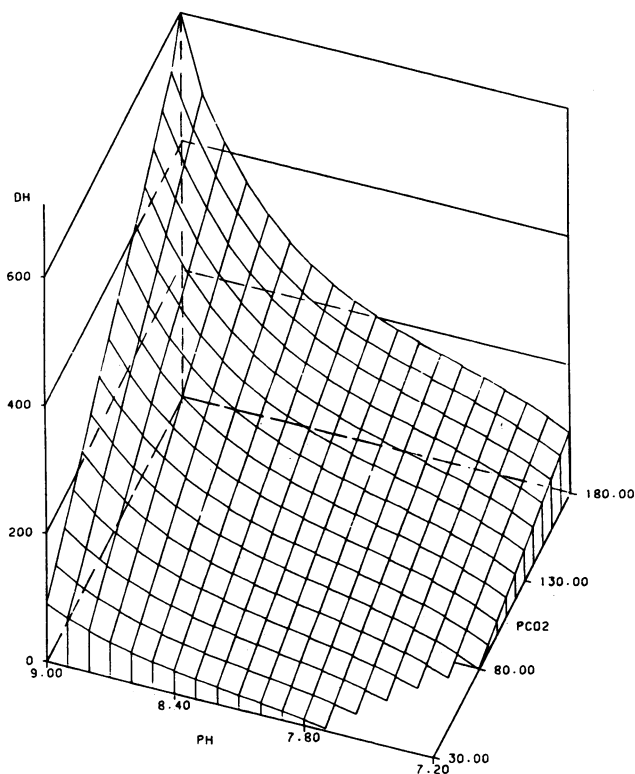


Figure 1. Computer model relating H^+ secretion (DH) to intracellular PCO_2 and pH.

mmHg; (2) tubular PCO_2 is identical to arterial PCO_2 , i.e., 120 mmHg. The calculated rates of absolute proximal bicarbonate reabsorption under these conditions are $679 \text{ pmol} \cdot \text{min}^{-1}$, at a PCO_2 of 180 mmHg, and $621 \text{ pmol} \cdot \text{min}^{-1}$, at a PCO_2 of 120 mmHg.

Assuming that proximal bicarbonate reabsorption is mediated only by H^+ secretion, our model shows that in order to observe rates of H^+ secretion of the magnitude observed in group C, an intracellular pH over 9.00 at an intracellular PCO_2 of either 120 or 180 mmHg is required. Since such extremely alkaline intracellular pH values of over nine are unlikely, it is clear that during hypercapnia as well as normocapnia (27), an additional source of intracellular H^+ must exist to account for the observed rate of proximal bicarbonate reabsorption.

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