Effects of Chloride and Extracellular Fluid Volume on Bicarbonate Reabsorption along the Nephron in Metabolic Alkalosis in the Rat

Reassessment of the Classical Hypothesis of the Pathogenesis of Metabolic Alkalosis

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Abstract

Volume expansion has been considered essential for the correction of chloride-depletion metabolic alkalosis (CDA). To examine the predictions of this hypothesis, rats dialyzed against 0.15 M $NaHCO_3$ to produce CDA and controls, CON, dialyzed against Ringer-HCO_3 were infused with either 6% albumin (VE) or 80 mM non–sodium chloride salts (CC) added to 5% dextrose (DX) and studied by micropuncture. CDA was maintained in rats infused with DX. VE expanded plasma volume (25%), maintained glomerular filtration rate (GFR), but did not correct CDA despite increased fractional delivery of total CO_2 (tCO_2) out of the proximal tubule (36±2%) as compared with VE/CON (24±4%; $P < 0.05$). In contrast, CC corrected CDA despite volume contraction (−16%) and lower GFR than CC/CON; proximal tCO_2 delivery in CC/CDA (29±4%) did not differ from VE/CDA. CC was associated with an increment in tCO_2 excretion. The data strongly suggest that maintenance and correction of CDA are primarily dependent upon total body chloride and its influences on intrarenal mechanisms and not on the demands of sodium or fluid homeostasis.

Introduction

Chloride and volume depletion both usually accompany the chloride-depletion metabolic alkaloses (CDA)[1] that include several of the most common clinical forms of metabolic alkalosis (1, 2). Repletion of both of these deficits has been considered important for the correction of CDA (3, 4). The classical hypothesis for the pathophysiology of CDA states that volume expansion is the predominant, indeed, mandatory change that must occur for the correction of CDA (3). In this scheme of the intranephronal redistribution of fluid reabsorption (5), extracellular fluid (ECF) volume expansion effects a decrease in proximal tubule fluid reabsorption that, in turn, decreases the reabsorption of bicarbonate in this nephron segment. As a result, more bicarbonate is delivered to distal sites in the nephron, which are considered to have a lesser capacity to reabsorb bicarbonate, but a greater capacity to reabsorb chloride. Thus, bicarbonate is excreted; chloride, retained; and the alkalosis, corrected.

We have recently proposed and provided evidence for an alternative hypothesis in which the provision of chloride results in a series of intrarenal events that leads to complete correction of CDA without the need for expansion of the ECF volume (6–8). However, in these studies, sodium was administered concomitantly with chloride, although without evidence for volume expansion. Furthermore, the effect of volume expansion without chloride repletion and changes in bicarbonate delivery to the distal nephron during correction were not examined. Thus, the basic tenets of the classical hypothesis were not excluded in those studies.

The purpose of the present studies, then, was to examine the principal predictions of the classical hypothesis for the pathophysiology of CDA with two diametrically opposed protocols: chloride administration with unequivocal sustained volume contraction and volume expansion without chloride administration. To accomplish this objective, we produced acute stable CDA by peritoneal dialysis (PD) as before (9) and infused both control and alkalotic rats with solutions containing either albumin or chloride in a concentration less than that in plasma and without sodium. During these infusions, fluid, chloride, and total CO_2 (tCO_2) handling by segments of superficial cortical nephrons were examined.

Methods

General. Male Sprague-Dawley rats that were obtained from either Charles River Breeding Laboratories, Inc. (Wilmington, MA) or Taconic Farms, Inc. (Germantown, NY) ate regular rat chow (Ralston-Purina Co., St. Louis, MO) and drank tap water ad lib. before all studies; body weights (BW) ranged from 235 to 335 g. On the day of dialysis, rats were anesthetized with an intraperitoneal injection of Inactin (Promonta, Hamburg, Federal Republic of Germany), 100 mg/kg BW. The animals were placed on a heated table and their body temperatures were maintained at 37°C. The femoral artery was catheterized with PE-50 tubing and an arterial blood sample obtained for hematocrit. This and subsequent blood samples were replaced quantitatively by volume with a suspension of erythrocytes that were obtained from a littermate bled by aortic puncture. Blood so obtained was centrifuged and the supernatant plasma removed. The erythrocytes were resuspended in 5% dextrose with 6% bovine serum albumin (BSA) (Sigma Chemical Co., St. Louis, MO) to a packed cell volume of 45%. The resultant suspension was gassed slowly with 95% O_2 and 5% CO_2 in a 37°C-water bath.

A tracheostomy and two PE-50 jugular venous catheters were placed. Warmed dialysate (15 ml/100 g BW) was infused into the peritoneal

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1. *Abbreviations used in this paper:* BW, body weight; CDA, chloride-depletion metabolic alkalosis; ECF, extracellular fluid; GFR, glomerular filtration rate; PD, peritoneal dialysis; SNGFR, superficial nephron GFR; tCO_2, total CO_2.

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cavity through an 16-gauge catheter. Control rats, CON, were dialyzed against Ringers-HCO_3 solution. In alkalotic rats, CDA was produced by a dialysate composed of 150 mM Na^+, 4 mM K^+, 154 mM HCO_3^-, and 15 g/liter glucose. After 30 min, the dialysate was drained. Previous studies have shown that this CDA dialysate produces a negative chloride balance of 1,500–2,000 μeq and neutral or slightly positive sodium and potassium balance (7). To correct for dialysis-related fluid shifts, 6% BSA in 5% dextrose was infused slowly in a volume of 1.0 ml/100 g BW for each 5.5% change in hematocrit (7).

All rats were then infused with one of several maintenance solutions described below for the remainder of the experiment. The bladder was catheterized with PE-50 tubing. In all experiments, the left kidney was exposed through a subcostal incision and placed in a Lucite cup. When the peritoneal cavity was entered, an undetermined small volume of fluid often leaked out in both groups. Neither this fluid—most likely, a remainder from the hypertonic dialysate—nor surgical fluid losses were replaced.

Experimental protocol. Five groups of rats were studied initially. First, to establish the stability of this model of acute CDA, group DX/CDA was infused with only 5% dextrose in water (infusion DX) at 0.6 ml/h/100 g BW.

To assess the effect of chloride administration in the setting of sustained volume contraction, CC/CON and CC/CDA groups were infused with 5% dextrose in water containing 80 mM Cl^-, 20 mM K^+, 20 mM Li^+, 10 mM Ca^{++}, and 10 mM Mg^{++} (infusion CC) at 0.6 ml/h/100 g BW. Four different cations were used to avoid any undesirable effects that might be associated with high plasma concentrations of any of them. After the 4-h CC infusions, plasma Li^+, Ca^{++}, and Mg^{++} concentrations did not differ (P = NS): 0.4±0.1 meq/liter CON and 0.5±0.1 meq/liter CDA; 9.9±0.1 mg/dl CON and 9.9±0.1 mg/dl CDA; and 2.9±0.2 mg/dl CON and 2.6±0.3 mg/dl CDA, respectively.

To assess the effect of the expansion of the ECF volume alone, VE/CON and VE/CDA groups were infused with 5% dextrose in water containing 6% BSA (infusion VE) at 2.5 ml/h/100 g BW.

To show that the administration of chloride to CDA rats induces an increment in urinary tCO_2 excretion during either volume expansion or contraction and that the volume expansion protocol did not prevent correction of CDA, two additional protocols were undertaken. In the volume expansion series, the aforementioned combination of non-sodium chloride salts was added to the VE infusate in concentrations of 20 mM (n = 6) or 80 mM (n = 4) in an additional group, VC/CDA; the former chloride concentration approximated the load and the latter the concentration given to the VE/CDA group. In the volume contraction series, CDA rats were switched to the CC infusion after 2 h of the DX infusion (CC/CDA–1 group; n = 6) or continued on the DX infusion (DX/CDA–1 group; n = 6). Only blood composition and urinary anion excretion were determined in both of these protocols.

Finally, to confirm that the correction of CDA occurred by a renal mechanism, separate CC/CDA rats (n = 6) were subjected to functional bilateral nephrectomy by occlusion of the renal pedicles immediately after completion of dialysis as previously described (8). After a 3-h infusion of the CC solution an aortic blood sample was obtained for determination of plasma chloride and tCO_2 concentrations. To control for the possibility that the several cations in the CC infusate were exchanged for intracellular hydrogen ion or for any corrective effect of functional nephrectomy per se, separate DX/CDA rats (n = 6) were studied in a similar manner with 3-h infusions of the DX solution.

Infusion studies. After the preparative surgery, which required ~ 60 min, the intravenous infusions that also included 4% polyfructosan were continued for an additional 180 min, in the final 60 min of which inulin clearances were determined. Blood samples were obtained for inulin, chloride, and tCO_2 determinations at the beginning and end of this clearance period. A urine collection was obtained for the determination of volume and inulin concentration. Each experiment was terminated with an aortic puncture for determination of pH and plasma sodium, potassium, chloride, and tCO_2 concentrations.

Free-flow micropuncture studies. Latest proximal and earliest distal tubule puncture sites were selected by intraluminal injections of 7.5% Food, Drug, and Cosmetic Green (Keystone Aniline & Chemical Co., Chicago, IL) with a 3-μm tip pipette. 67 μCi/ml, [Methoxy-^{3}H]inulin, which had been dialyzed against distilled water for 72 h before use, was added to the maintenance infusion. After 1.5 h of the maintenance infusion, one to three of each early distal timed and late proximal untimed, unblocked tubule-fluid samples were collected during the subsequent 60–90 min, and a timed urine sample were obtained for measuring volume [^{3}H]inulin activity, and sodium, potassium, chloride, and tCO_2 concentrations. Volume, [^{3}H]inulin activity, and tCO_2 and chloride concentrations were determined for each tubule fluid sample. At the beginning, midpoint, and end of the clearance period arterial blood samples were obtained for measuring hematocrit, [^{3}H]inulin activity, and tCO_2 and chloride concentrations. A final arterial sample was obtained for measuring hematocrit, pH, and sodium, potassium, chloride, tCO_2, and protein concentrations. In DX/CDA group only late proximal punctures were obtained and those, only for [^{3}H]inulin activity.

Analytical techniques. Activities of [^{3}H]inulin in tubule fluid, plasma, and urine were determined by liquid–scintillation counting (Tracor Analytic 6892; TM Analytic, Inc., Elk Grove, IL). Corrections were made for background activity. Quench corrections were obtained from an internal standard. The volumes of tubule fluid samples were measured in constant-bore glass tubing with a microslide comparator (Gaertner Scientific Co., Chicago, IL). Chloride concentrations in tubule fluid were determined by the second method of Ramsay et al. (10). tCO_2 concentrations in plasma, urine, and tubule fluid were determined by microcalorimetry (Picapnotherm; World Precision Instruments, Inc., New Haven, CT) (11). Sample size varied between 10 and 15 nl; the intraassay coefficient of variation in our laboratory is 2.15 for the 25-mM standard.

Inulin concentrations in urine and plasma in the 4-h infusion studies were determined by the anthrone method. Sodium and potassium concentrations in urine and plasma were determined by flame photometry (model 943; Instrumentation Laboratory, Inc., Lexington, MA) and chloride concentrations by electrometric titration (Buchler Instruments, Inc., Fort Lee, NJ). Arterial pH was determined with a BMS3 MK2 blood gas analyzer (Radiometer A/S, Copenhagen, Denmark). Protein concentrations were determined by refractometry (model 10400A; American Optical Corp., Instruments Div., Buffalo, NY).

Calculations. Whole kidney glomerular filtration rate (GFR) and urinary excretions of sodium, potassium, tCO_2, and chloride were calculated according to standard expressions. Plasma anion gap was estimated by the difference between the plasma sodium concentration and the sum of the plasma chloride and tCO_2 concentrations. The various single nephron functional characteristics including superficial nephron GFR (SNGFR), absolute and fractional reabsorptions, deliveries of fluid, tCO_2, and chloride from both proximal and distal puncture sites, and changes in plasma volume were calculated as previously described (12); the loop segment refers to that portion of superficial cortical nephrons between the latest surface proximal convolution and the earliest surface distal convolution and includes the proximal straight tubule, the thin descending limb, and the medullary and cortical portions of the thick ascending limb of Henle's loop. Ultrafiltrate tCO_2 and chloride concentration were obtained by multiplying the plasma concentration by factors of 1.15 and 1.06, respectively (13, 14). Average values for each animal for all single nephron functional variables were used to calculate group means. Absolute proximal reabsorptions were estimated from the product of the mean SNGFR determined from early distal punctures in each animal and the fractional reabsorption, from each proximal puncture site. Fractional deliveries are expressed as a percentage of filtered load.

Values given are mean±SEM. The 4-h infusion studies were carried out in random order in the five groups; statistical significance between these groups was assessed by Duncan's multiple range test. In the micropuncture studies, CON and CDA groups were studied contemporaneously for each type of infusion; statistical significance was assessed between these groups with Student's unpaired t test. Comparisons within any group were made with Student's paired t test (15). Significance was set at the 5% level.

Results

Systemic effects. After the induction of alkalosis by PD, the infusion of 5% dextrose produced no change in the plasma chloride or tCO_2 concentrations in DX/CDA rats after 4 h of infusion (Fig. 1, *left* and *center;* Table I). Similarly, neither the CC nor VE infusion changed plasma chloride or tCO_2 concentrations in either CON group. In VE/CDA group, the plasma tCO_2 concentration was also unchanged, but the plasma chloride concentration decreased, probably due to dilution associated with impaired urinary water excretion (12, 16). In contrast to these four groups, plasma chloride concentration increased and that of tCO_2 decreased reciprocally during the 4-h infusion in CC/CDA group. Similarly, in VC/CDA group that received 20 mM chloride, plasma chloride concentration increased 5.9±1.6 meq/liter ($P < 0.01$) despite a 6.5±1.4 meq/liter decrease ($P < 0.005$) in plasma sodium concentration; plasma tCO_2 concentration decreased reciprocally by 7.3±0.9 meq/liter ($P < 0.001$). Thus, the degree of correction was similar to that in CC/CDA group. The hyponatremia in this group lends support to our view that dilution explains the decrease in plasma chloride concentration in VE/CDA group. VC/CDA group that received 80 mM chloride corrected alkalosis completely in 4 h: plasma chloride 103±1 meq/liter and tCO_2 26.5±0.7 meq/liter.

The essential role of the kidney in the correction of CDA is shown in the studies conducted after functional bilateral nephrectomy. In that CC/CDA group, unlike those with intact kidneys, plasma chloride (from 78.7±1.1 to 79.2±11.2 meq/liter, change 0.5±0.5 meq/liter, P = NS) and tCO_2 (from 39.6±1.7 to 39.0±1.0 meq/liter, change −0.7±0.8 meq/liter, P = NS) concentrations did not change or differ (P = NS) from those in DX/CDA group treated similarly (from 76.9±0.3 to 76.7±0.8 meq/liter Cl^-, change 0.3±0.6 meq/liter, P = NS; from 39.5±0.4 to 39.5±0.4 meq/liter tCO_2, change 0.0±0.4 meq/liter, P = NS). Thus, correction of alkalosis occurred by a renal mechanism. Furthermore, an acidosis due to the exchange of the several cations infused in CC/CDA group for intracellular hydrogen ion is excluded by comparison with the results in DX/CDA group that also maintained alkalosis.

After 4 h of infusion, plasma volume as assessed by arterial hematocrits (Fig. 1, *right:* Table I) was decreased in DX/CDA (−24.9%), CC/CDA (−16.3%), and CC/CON (−20.3%) groups and increased in VE/CDA (25.4%), VC/CDA (37.5%), and VE/CON (21.3%) groups. Plasma protein concentrations after the VE infusions that contained BSA did not differ between VE/CON (6.8±0.2 g/dl) and VE/CDA (7.1±0.2 g/dl) groups; those after CC infusion (CC/CON [5.6±0.3 g/dl]; CC/CDA [5.5±0.1 g/dl]) or DX infusion (DX/CDA [5.7±0.2 g/dl]) also did not differ (P = NS).

Fluid handling. Experimental kidney GFR determined during the 4th h of infusion was lowest in CC/CDA group, in which the alkalosis was correcting, and 26% lower than that in CC/CON group, although was statistically ($P < 0.05$) lower as compared with only VE/CDA group (Table I). Urinary flow rates were about 10-fold higher in the VE groups.

During micropuncture in the 3rd h (Table II), experimental kidney GFRs did not differ with either infusion. However, the GFR in CC/CDA group was again 20% lower than that in CC/CON group. Urinary sodium and potassium excretion rates were lower in both CC groups as compared with the VE groups but not between CON and CDA groups with either infusion.

SNGFR was decreased only in group CC/CDA in keeping with the experimental kidney GFRs (Tables I–II). Similar to experimental kidney GFR, SNGFR in CC/CDA group was 22% lower than that in CC/CON group. Absolute and fractional fluid handling in the proximal convolutions and loop segments did not differ (Table III). In keeping with the most pronounced plasma volume contraction in DX/CDA group (−24.9%), fractional proximal fluid reabsorption (68.2±3.4%) was highest in this group and higher ($P < 0.005$) than that in VE/CDA group, thereby demonstrating that an increase in fluid delivery out of the proximal tubule was achieved in VE/CDA.

Chloride handling. Filtered chloride loads in superficial nephrons were decreased in both CDA groups: 21% with VE

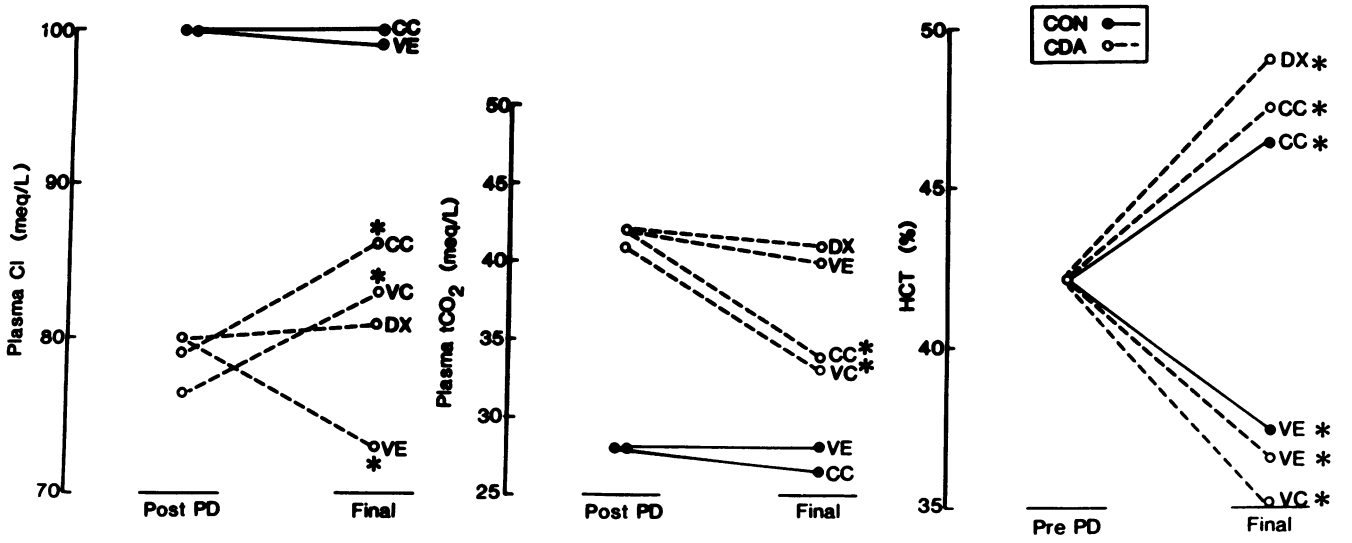

Figure 1. The effects of dialysis and 4-h fluid infusions on plasma chloride concentration (*left*), plasma tCO_2 concentration (*center*), and arterial hematocrit (*right*). Mean values are given. *$P < 0.05$.

Table I. Final Blood Composition and Renal Function after 4-h Infusions

Group	Plasma					Arterial blood		Inulin clearance	Urinary flow rate
	Na^+	K^+	Cl^-	tCO_2	Anion gap	pH	Hct		
	meq/liter	*meq/liter*	*meq/liter*	*meq/liter*	*meq/liter*		%	*μl/min/100 g BW*	*μl/min*
VE/CON (n = 6)	139±3	4.0±0.2	100±1	28±1	12±3	7.40±.01	37.4±0.9	723±54	131±14
VE/CDA (n = 6)	138±2	3.1±0.1*‖¶	73±1*‖¶	40±1*‖	25±2*‖¶	7.53±.01*§	36.6±0.5	874±69	98±16
CC/CON (n = 6)	135±1‖	4.7±0.2	99±1	27±1	7±1	7.42±.02	47.6±0.8*‡	766±108	7±1*‡
CC/CDA (n = 6)	144±1	4.4±0.1	86±1*§	34±1*§	23±2*‖¶	7.51±.02*§	46.4±0.9*‡	570±54‡	7±1*‡
DX/CDA (n = 6)	138±3	3.8±0.2‖	81±1*‖	41±2*‖	16±4§	7.59±.02*§	49.1±0.5*‡	650±89	10±3*‡

$P < 0.05$ compared with: *VE/CON, ‡VE/CDA, §CC/CON, ‖CC/CDA, and ¶DX/CDA.

infusions ($P < 0.05$) and 36% with CC infusions ($P < 0.005$) (Table IV). However, resultant significant decreases in absolute chloride handling were detectable in only a few instances: delivery to and reabsorption in the loop segment in VE/CDA group and delivery to the distal convolution in CC/CDA group. In contrast, no differences in fractional chloride handling in the proximal convolution or the loop segment were observed in CON and CDA groups with either infusion. Tubule fluid chloride concentrations at the late proximal and early distal sites were lower in both CDA groups (Table V). As expected, urinary chloride excretions were low in the CDA groups (Table II).

Bicarbonate handling. The filtered tCO_2 load in VE/CDA group was increased because volume expansion presumably (17) abrogated a decrease in SNGFR, which had previously been seen in CDA (7, 18) (Table VI). In the proximal convolution, absolute tCO_2 reabsorptions did not differ between the CON and CDA groups with either infusion. In the loop segment, absolute tCO_2 reabsorption, although ~ 100 pmol/min greater in both VE/CDA and CC/CDA groups, did not differ from controls; fractional reabsorption was greater in VE/CON than in VE/CDA group. However and most importantly, both fractional and absolute deliveries of tCO_2 out of the late proximal and the early distal tubules were increased in both VE/CDA and CC/CDA groups. Tubule fluid tCO_2 concentrations at the late proximal and early distal sites were higher in both CDA groups (Table V).

Urinary tCO_2 excretions were higher, but not significantly so ($P > 0.05 < 0.1$), in both CDA groups as compared with their appropriate CON groups during the micropuncture period (Table II). However, urinary tCO_2 excretion during volume contraction in CDA increased promptly by 321±112 neq/min ($P < 0.001$) when the DX infusion was switched to the CC infusion (CC/CDA-1 group), whereas it decreased by 112±53 neq/min ($P < 0.1 > 0.05$) when the DX infusion was continued (DX/CDA-1 group) (Fig. 2). In CC/CDA-1 group, plasma chloride increased (+3.0±0.8 meq/liter, $P = 0.0024$) and plasma tCO_2 decreased (−5.6±1.6, $P = 0.024$) over 90 min; in DX/CDA-1 group, neither plasma chloride (0.2±0.8, P = NS) nor plasma tCO_2 concentration (−1.8±0.9, P = NS) changed. Furthermore, compared with VE/CDA group, urinary tCO_2 excretion increased to 671±158 (P = NS), when 20 mM chloride was infused during volume expansion in VC/CDA group, and to 1,287±87 neq/min/100 g BW ($P < 0.05$), when 80 mM chloride was infused.

Absolute tCO_2 reabsorption in the proximal convolution was closely associated ($P < 0.01$) with filtered tCO_2 load (Fig. 3). The reabsorptive rate at any filtered tCO_2 load for the CON groups was higher than ($P = 0.0018$) that for the CDA groups; these relationships did not differ within control or alkalotic groups for either the CC or VE infusion. Absolute tCO_2 reabsorptions were also associated ($P < 0.01$) with SNGFR (CON, $Y = 23.72+1.17\ X$; CDA, $Y = 28.20+1.16\ X$).

Absolute tCO_2 reabsorption in the loop segment was also positively correlated with absolute tCO_2 delivery. The correlation coefficient was 0.989 for both CON groups ($P < 0.001$) and 0.878 for both CDA groups ($P < 0.001$). As in the proximal convolution, the reabsorptive rate for tCO_2, as defined by the slope, was lower for any delivery rate in CDA rats (0.658±0.027) than in CON rats (0.841±0.025; $P < 0.0003$).

By inspection of the data for the CDA groups, it is clear that correction of alkalosis in CC/CDA proceeds in face of a lower SNGFR and is not dependent upon increased delivery of tCO_2

Table II. Experimental Kidney Functions during Micropuncture

Group	Inulin clearance	Urinary excretion			
		Na^+	K^+	Cl^-	tCO_2
	µl/min/100 g BW	*neq/min/100 g BW*	*neq/min/100 g BW*	*neq/min/100 g BW*	*neq/min/100 g BW*
VE/CON*	505±54	92±22	293±68	210±72	130±79
VE/CDA*	467±44	362±77‡	817±69§	48±15	597±277
CC/CON‖	610±88	40±21	208±38	145±38	11±5
CC/CDA‖	486±52	112±63	510±79§	9±2§	50±31

*Data for VE infusion. ‡$P < 0.05$. §$P < 0.001$. ‖Data for CC infusion.

beyond the early distal convoluted tubule since the latter was also present in VE/CDA group that did not correct. Furthermore, because the deliveries of chloride and tCO_2 were, if anything, greater in VE/CDA group as compared with CC/CDA, insufficient delivery of these anions beyond the early distal convoluted tubule cannot explain the failure of those segments to respond in a manner that permits correction of alkalosis.

Discussion

Acute CDA induced by dialysis in this model was stable over 4 h when rats were infused with 5% dextrose; volume contraction also occurred. When 6% BSA was added and infused at a fourfold higher rate, plasma volume was expanded, GFR was increased, and fractional proximal fluid reabsorption decreased, but CDA was not corrected. Plasma anion composition remained stable also in CON rats that had a normal arterial pH. In contrast, when 80 mM chloride was added as non-sodium salts even at the low rate of infusion, CDA was corrected progressively; plasma anion composition again remained stable in the CON group. This correction of CDA occurred despite both sustained contraction of the plasma volume, similar to that seen in DX/CDA group, and a persistent decrease in GFR. Also, any effect of the VE infusion that might have precluded correction was excluded by VC/CDA group in which only 20 mM chloride added to the VE infusate lead to correction. Indeed, the addition of 80 mM chloride to the VE infusate lead to complete correction within 4 h. Finally, the failure of chloride to correct CDA after functional bilateral nephrectomy in CC/CDA group establishes that correction occurs by a renal mechanism as we have previously shown in a similar model (8).

In both CDA groups, fractional and absolute deliveries of tCO_2 out of the late proximal and the early distal tubules were increased, but correction proceeded only in the group infused with chloride. Correcting alkalosis was attended by intense chloride conservation and increased urinary excretion of tCO_2. However, more tCO_2 was also excreted in VE/CDA group in the setting of a massive diuresis, which confounds the interpretation. In response to a hypotonic medium, cells normally regulate volume primarily by the extrusion of KCl (19). Under conditions of intracellular chloride depletion, conceivably bicarbonate or its metabolic equivalent may move out of cells with potassium (19, 20) as intracellular volume is adjusted and, thus, may account for the maintained plasma tCO_2 concentration in the setting of bicarbonaturia. Notwithstanding this observa-

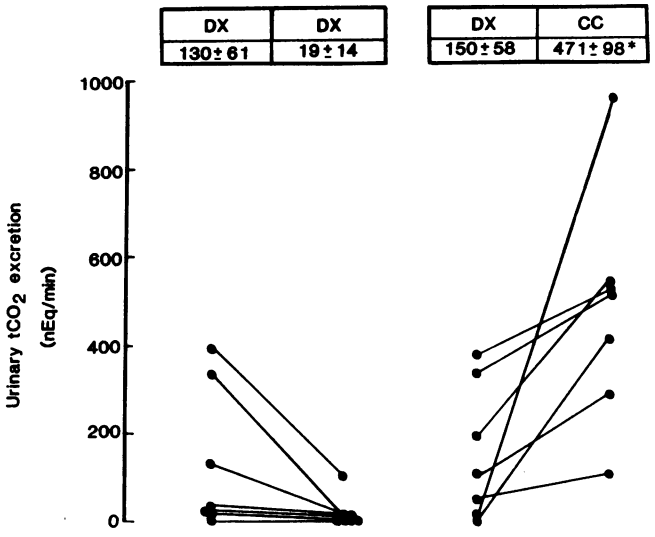

Figure 2. Effect of chloride administration on urinary tCO_2 excretion in the experimental kidney during CDA. The types of infusion and the mean urinary tCO_2 excretions are indicated in each column above the graph of data points.

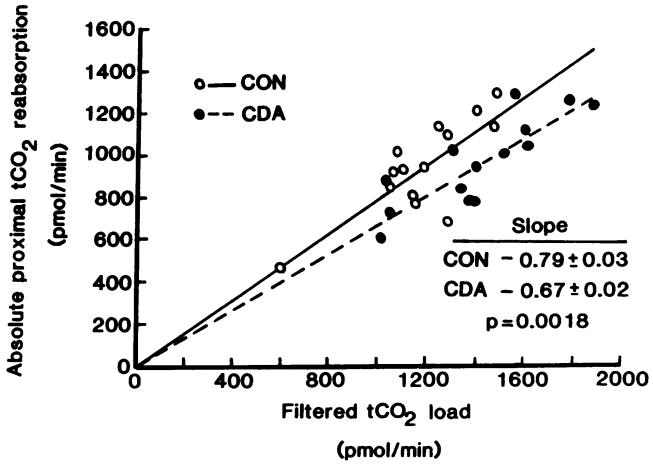

Figure 3. Relationship between filtered tCO_2 load and absolute tCO_2 reabsorption in the proximal convoluted tubule. The regression lines for all control (—) and all alkalotic (---) rats are given and necessarily passed through the origin in this analysis.

Table III. Superficial Nephron Fluid Handling

Group	Filtered load	Proximal		Loop segment				Early distal	
		Reabsorption		Delivery		Reabsorption		Delivery	
	nl/min	*nl/min*	%	*nl/min*	%	*nl/min*	%*	*nl/min*	%
VE/CON‡ (n = 8)	37.6±3.2	20.4±1.8	55.8±2.5	17.3±2.0	45.5±2.5	11.4±1.5	65.5±3.5	5.9±0.8	15.8±1.8
VE/CDA‡ (n = 6)	37.6±1.9	21.6±1.2	57.3±1.3	16.2±1.0	42.6±1.3	9.8±0.9	60.7±3.8	6.4±0.7	16.7±1.7
P‡	NS	NS	NS	NS	NS	NS	NS	NS	NS
CC/CON§ (n = 9)	38.2±2.7	24.3±2.5	63.3±4.5	13.9±1.8	36.6±4.5	8.1±1.5	56.3±4.5	5.8±0.7	15.0±1.4
CC/CDA§ (n = 6)	30.0±2.0	20.6±3.4	58.8±3.2	11.6±0.7	41.2±3.2	7.6±0.4	66.5±3.2	4.3±0.6	14.7±2.0
P§	<0.05	NS	NS	NS	NS	NS	NS	NS	NS

*Within loop segment. ‡Data for VE infusion. §Data for CC infusion.

Table IV. Superficial Nephron Chloride Handling

Group	Filtered load	Proximal		Loop segment				Early distal	
		Reabsorption		Delivery		Reabsorption		Delivery	
	pmol/min	*pmol/min*	%	*pmol/min*	%	*pmol/min*	%*	*pmol/min*	%
VE/CON‡ (n = 8)	3813±338	1768±182	48.0±2.5	2044±174	51.9±2.5	1840±157	90.1±2.0	204±45	5.4±1.0
VE/CDA‡ (n = 6)	3008±146	1500±110	50.0±2.7	1507±118	50.0±2.7	1335±101	88.9±2.1	172±38	5.2±1.2
P‡	<0.05	NS	NS	<0.05	NS	<0.025	NS	NS	NS
CC/CON§ (n = 9)	3994±298	2003±325	56.0±6.0	1733±243	44.0±6.0	1470±242	83.0±3.9	263±49	6.7±1.2
CC/CDA§ (n = 6)	2553±153	1352±213	52.1±5.3	1184±110	47.9±5.3	1073±93	90.8±0.9	130±25	5.0±0.9
P§	<0.005	NS	NS	NS	NS	NS	NS	<0.05	NS

*Within loop segment. ‡Data for VE infusion. §Data for CC infusion.

Table V. Tubule Fluid Chloride and tCO_2 Concentrations

Group	Late proximal		Early distal	
	Cl^-	tCO_2	Cl^-	tCO_2
	meq/liter	*meq/liter*	*meq/liter*	*meq/liter*
VE/CON*	120±3	13.8±0.9	34±3	6.9±0.9
VE/CDA*	97±3‡	30.2±1.1‡	28±5	29.8±1.8‡
CC/CON§	125±3	10.4±0.9	44±4	8.3±1.0
CC/CDA§	106±2‡	26.1±1.5‡	29±2‡	23.5±3.6‡

*Data for VE infusion. ‡$P < 0.001$. §Data for CC infusion.

tion, the effect of chloride administration to correct alkalosis by a renal mechanism is clearly seen during both volume contraction and expansion. These data show that the kidney in VE/CDA group is unable to lower plasma tCO_2 concentration by adequate renal excretion of bicarbonate or base equivalents despite massive volume expansion because of the unavailability of additional exogenous chloride.

Role of glomerular filtration rate. CDA can, a priori, be maintained by either a decrease in the filtered load of bicarbonate or an increase in the reabsorption of bicarbonate or both. A decrease in GFR effecting a decrease in filtered bicarbonate load may occur as a consequence of volume contraction, at least in the acute setting (21) or, as we have recently proposed (18), tubuloglomerular feedback. The importance of a decrease in GFR for the maintenance of CDA or an increase for its correction has not been established and the available data conflict. Kassirer and co-workers showed that no changes occur in inulin clearance during the induction of metabolic alkalosis by gastric drainage (22) or $NaNO_3$ infusion (23). In contrast, Berger et al. (24) showed that a decrease occurs in inulin clearance during induction by gastric drainage in men on a low sodium diet. We have recently reported that, in man, GFR decreases with the generation of CDA but does not subsequently increase with correction when negative sodium balance is maintained (25).

In the dog, the correction of metabolic alkalosis, which had been induced by gastric drainage, was not attended by a change in inulin clearance (26). However, in the rat, Cogan and Liu (21) showed that a markedly decreased SNGFR returned to normal after correction of CDA by the infusion of large volumes of isotonic NaCl. Maddox and Gennari (27) also noted a lower SNGFR and kidney GFR during the 1st wk of chronic alkalosis, but more recently they (13) have reported a higher SNGFR in diuretic-induced CDA of 2–4 wk duration as compared with controls. We have previously observed a decrease in SNGFR in our model of acute CDA induced by PD and have provided evidence that this decrease was related not to volume contraction, but rather to tubuloglomerular feedback (18). Whether GFR increases because of the correction of alkalosis or an increased GFR initiates correction was not established by our previous study. Recently, Cogan (28) has argued that an increase in GFR alone, produced by the infusion of atrial natriuretic factor, corrects chronic metabolic alkalosis in the rat; however, the vehicle, which included chloride, was not infused as a necessary control in that study.

In the present study, correction of CDA proceeded despite a maintained lower GFR, sustained contraction of ECF volume, and negative sodium balance in CC/CDA group. The well-maintained SNGFR in the VE/CDA group is probably related to the effect of volume expansion to blunt or abolish tubuloglomerular feedback (17); volume expansion per se seems an unlikely explanation since SNGFR in VE/CON group was similar to that in CC/CON group. However, even this well-maintained SNGFR with an increase in the filtered load of tCO_2 did not lead to correction of CDA without chloride administration. We have also recently shown correction of CDA by non-sodium chloride salts despite a persistently reduced GFR in chronic CDA in the rat (29). Thus, it appears that the reduction in GFR in our acute model of CDA is secondary to the alkalosis and, as we have previously proposed, serves to lessen the delivery of sodium with bicarbonate to the distal nephron, and thereby to blunt the magnitude of the volume depletion that often accompanies alkalosis especially during the acute disequilibrium stage. Since alkalosis can be corrected despite the persistence of this low GFR and not with restoration of GFR alone, we conclude that a reduced GFR is not essential to the maintenance of CDA.

Bicarbonate reabsorption in the proximal convoluted tubule. In the present study, absolute tCO_2 reabsorption in the proximal convoluted tubule was similar in CON and CDA groups whether volume contracted or expanded and whether maintaining or correcting CDA (Table VI) and, as previously shown by Cogan and Liu (21) and Maddox and Gennari (27), was positively correlated with the filtered tCO_2 load (Fig. 3). Maddox and Gennari observed that this relationship in chronically alkalotic (2–4 wk) rats with high SNGFRs was not different from normal controls (13), but noted that proximal tCO_2 reabsorption in several models of acute CDA in the rat (21, 30) appears to be decreased with respect to filtered load compared with normals. ECF-volume expansion, which was also present in these models, primarily influences NaCl rather than $NaHCO_3$ reabsorption in this segment (31), although there is disagreement regarding the extent of this difference (32). Nevertheless, in the present study, acutely alkalotic rats showed a decreased reabsorptive rate for tCO_2 in the proximal convoluted tubule for any given filtered load as compared with the appropriate CON group, whether volume expanded or contracted. Thus, volume expansion is an unlikely explanation for the lower reabsorptive rate for bicarbonate, whereas an influence of peritubular bicarbonate concentration to inhibit proximal bicarbonate reabsorption may explain it (33, 34). Whatever the mechanism, changes in bicarbonate handling in the proximal convoluted tubule do not appear to explain the correction.

The cause for the difference in reabsorptive rates for bicarbonate between the acute and chronic alkalotic states is not apparent. The insertion of hydrogen ion secretory pumps, potassium depletion (34), and proximal tubule hypertrophy (13) are all possible explanations for enhanced proximal bicarbonate reabsorption in chronic alkalosis despite the persistently elevated peritubular bicarbonate concentration.

Bicarbonate reabsorption in the loop segment. Absolute net tCO_2 reabsorption in the loop segment of these superficial cortical nephrons was not significantly greater in alkalotic rats, whether correcting or maintaining CDA, than that in CON groups. Fractional tCO_2 reabsorption in VE/CDA group was actually lower than that in its CON. Thus, changes in bicarbonate handling in the loop segment do not appear to participate in the correction of CDA.

In CC/CDA group, which is the closest approximation of a normal control group that we studied, absolute tCO_2 reabsorption was comparable to that reported by Bichara et al. (32) and

Table VI. Superficial Nephron tCO_2 Handling

Group	Filtered load	Proximal		Loop segment				Early distal	
		Reabsorption		Delivery		Reabsorption		Delivery	
	pmol/min	*pmol/min*	%	*pmol/min*	%	*pmol/min*	%*	*pmol/min*	%
VE/CON‡ (n = 8)	1145±104	853±90	75.9±3.9	292±63	24.1±3.9	270±62	82.7±3.5	49±9	5.3±0.9
VE/CDA‡ (n = 6)	1569±79	1007±68	63.9±1.8	516±27	36.1±1.8	361±25	64.5±3.9	200±25	14.2±2.0
P‡	<0.01	NS	<0.05	<0.025	<0.05	NS	<0.01	<0.01	<0.005
CC/CON§ (n = 9)	1118±91	959±91	83.0±2.8	192±33	17.0±2.8	146±35	71.0±6.2	45±7	5.0±0.8
CC/CDA§ (n = 6)	1282±94	909±89	70.8±3.9	372±54	29.2±3.9	257±41	69.9±4.3	114±22	10.5±2.3
P§	NS	NS	<0.02	<0.025	<0.025	NS	NS	<0.005	<0.05

* Within loop segment. ‡Data for VE infusion. §Data for CC infusion.

higher than the 34 pmol/min calculated by Buerkert et al. (35); fractional delivery in this group was higher than the 12.2% determined by DuBose et al. (36) for their normal controls. Since the tCO_2 or HCO_3 concentrations in late proximal and distal tubule fluid in our study were comparable to all of these previous reports, higher tubule fluid-flow rates in our study can account for the difference from the studies of Buerkert et al. and DuBose et al. that were carried out in young, smaller Munich-Wistar rats. Based on isolated, perfused tubule studies, bicarbonate reabsorption in the thick ascending limb in Sprague-Dawley rats has been estimated to be between 20 and 36 pmol/min by Good et al. (37). Assuming a normal delivery rate of 200–300 pmol/min to the loop segment for the adult Sprague-Dawley rat, the majority of the net tCO_2 reabsorption probably occurs in the pars recta, which reabsorbs tCO_2 actively (38), or, less likely, in the descending thin limb. Thus, for any given delivery rate the lower tCO_2 reabsorptive rate to the loop segment observed in the alkalotic rats is consistent with our observations in the proximal convoluted tubule, although we cannot exclude a similar effect in the other included segments.

Anion reabsorption in the distal nephron. The classical hypothesis for the correction of CDA predicts that ECF-volume expansion decreases fluid and bicarbonate reabsorption in the proximal tubule, thereby increasing delivery of both to the distal nephron. Because these distal segments have been considered to possess a limited capacity to reabsorb bicarbonate, but a substantial capacity to reabsorb chloride, this increased delivery would theoretically permit correction by the selective retention of chloride and the enhanced excretion of bicarbonate. Our data suggest that the loop segment does not participate in the correction in this model. Despite increased delivery of tCO_2 out of the proximal convoluted tubule and the loop segment to the distal nephron, correction does not proceed without the administration of chloride. Rather, it appears that, in the absence of chloride administration, the distal convoluted tubule and the collecting duct segment continue to reabsorb sufficient bicarbonate to maintain the alkalosis.

At least two sodium-independent processes may importantly influence chloride and bicarbonate transport in the collecting duct: secretion of bicarbonate by a luminal chloride–bicarbonate exchange process in the cortical collecting tubule (39–41) and electrogenic proton secretion facilitated by chloride movement from the interstitium into the lumen in the outer medullary portion (42). In each of these, increasing luminal chloride concentrations could plausibly increase renal bicarbonate excretion. However, absolute delivery of chloride out of the early distal tubule was, if anything, less in the group that corrected (CC/CDA) as compared with the group that maintained (VE/CDA) CDA. Chloride concentrations in early distal tubule fluid were nearly identical in both of these groups. Furthermore, studies involving the volume-contracted groups, like CC/CDA-1, now in progress show similar delivery of chloride (136±17 peq/min CC; 133±16 peq/min DX; P = NS) and tCO_2 (174±18 peq/min CC; 187±33 peq/min DX; P = NS) to the early distal nephron even though bicarbonate excretion increases only in the group receiving chloride as in Fig. 2. We have also recently estimated that chloride reabsorption in the superficial distal convolution may be augmented during the correction of CDA (7), which would further decrease chloride delivery to the collecting duct. Thus, increased delivery of chloride to the collecting duct to facilitate net bicarbonate excretion in that segment is not

essential for correction. Taken together, these data strongly suggest that, in this model, key adjustments in anion excretion during the correction of CDA occur beyond Henle's loop and most likely in the collecting duct.

However, the manner by which the kidney perceives chloride administration remains elusive. In a different model of acute metabolic alkalosis, Beck et al. (43) have shown marked changes in intracellular electrolyte concentrations including a decrease in chloride from 20 to 11 mmol/kg wet wt in renal tubule epithelial cells. If intracellular chloride depletion[2] endows at least one cell type of the collecting duct or distal convolution with exquisite chloride conservation, a progressive increment in plasma chloride concentration might be brought about if such a transport mechanism retained virtually all chloride presented from either the basolateral or luminal aspect. Even though chloride is given at concentrations less than that in ambient plasma, mass balance would demand a progressive increase in both intracellular and extracellular chloride content. It is also possible that the ratio of luminal/cellular chloride concentration influences bicarbonate secretion in the cortical collecting tubule such that a slight increase in luminal chloride concentration facilitates bicarbonate excretion in the presence of lowered tubule cell chloride activity. In whatever manner the kidney recognizes the administration of chloride, the response appears to entail almost exclusively either the decrease of concomitant acid excretion or an increase in alkali excretion as bicarbonate and other unmeasured anions. Chloride appears to be intensely conserved during both maintenance and correction until the plasma chloride concentration is restored to normal or nearly so (18). How these transport mechanisms in the distal nephron are signalled and how their axial arrangement subsequently interacts to effect correction of alkalosis are important areas for further study.

The nature of unmeasured anions and their role in correction remains to be elucidated. For example, the magnitude of the bicarbonaturia in CC/CDA-1 group could account for ~ 20% of the decrement in plasma tCO_2 concentration based on the data in Fig. 2 and the observed decrement of 4 meq/liter in plasma tCO_2 in this group. This estimate assumes a fixed bicarbonate space of 0.4 BW, which could be an overestimate. However, unmeasured urinary anions,[3] estimated from the data in Table II, constituted 90% of excreted anions in CC/CDA group as compared with only 37% in CC/CON group. Metabolic alkalosis stimulates the production and excretion of organic anions of the tricarboxylic acid pathway (44, 45), and base excretion in the form of citrate has been shown to have an important role in diuretic-induced alkalosis in the rat (46). Although we do not know the nature of the unmeasured anions during either maintenance or correction in our model, the excretion of base equivalents could contribute importantly to the decrement in plasma tCO_2 concentration.

In summary, the present experiments show that chloride repletion alone corrects stable CDA, at least as produced in this model, and does so despite sustained volume contraction and a low GFR. Conversely, volume expansion and restoration of GFR without the concomitant administration of chloride does not correct CDA despite the delivery of copious amounts of bicarbonate to the distal nephron segments. The data in the present and previous experiments with this model do not support the classical hypothesis for the pathophysiology of the maintenance and correction of CDA. Rather, we interpret our data to indicate that chloride alone can correct CDA via intrarenal mechanisms residing principally in the collecting duct segment or, possibly, in juxtamedullary nephrons (47).

2. Our peritoneal dialysis-induced depletion of chloride clearly results in some intracellular chloride depletion because the plasma chloride concentration falls less than the calculated amount for the removed chloride (9), if one assumes an ECF volume of the rat of 20% of the BW.

3. Urinary anion gap = $([Na^+ + K^+) - (Cl^- + HCO_3^-)]$

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