Volume-independent Reductions in Glomerular Filtration Rate in Acute Chloride-Depletion Alkalosis in the Rat

Evidence for Mediation by Tubuloglomerular Feedback

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Abstract. We have recently described reduced superficial nephron glomerular filtration rate (SNGFR) in chloride-depletion alkalosis (CDA) without volume depletion. To elucidate the mechanism of this phenomenon, we studied three degrees of increasing severity of CDA (groups CDA-1, 2, and 3) produced by one or two peritoneal dialyzes against 0.15 M $NaHCO_3$ and electrolyte infusions of different Cl and HCO_3 content in Sprague-Dawley rats; control rats (CON) were dialyzed against and infused with Ringers-HCO_3. Extracellular fluid (ECF) volume was assessed by blood pressure, hematocrit, plasma protein concentration, and ^{125}I-albumin space; none of these variables differed among the four groups. Micropuncture of the latest proximal and earliest distal convolutions was carried out. As CDA intensified from CON to CDA-3 (plasma tCO_2 25±1 to 43±1 meq/L; $P < 0.01$), distally determined SNGFR declined progressively (40.9±1.7 to 28.3±1.8 nl/min; $P < 0.01$), while in early distal tubule fluid, flow rate (8.6±0.7 to 3.4±0.6 nl/min) and Cl concentration (36±2 to 19±3 meq/L) decreased and osmolality (110±5 to 208±12 mosmol/kg) increased ($P < 0.01$), and, in the loop segment, Cl reabsorption decreased progressively (2,009±112 to 765±128 peq/min; $P < 0.01$). In early distal tubule fluid, Cl concentration correlated positively and osmolality negatively with distally determined SNGFR ($P < 0.05$). Proximally determined SNGFRs did not differ among the four groups. Proximal tubule stop-flow pressure responses to increasing rates of orthograde perfusion of the loop segment from 0 to 40 nl/min did not differ between groups CON and CDA-2.

We interpret these data to show that reductions in SNGFR in CDA in the rat can occur by tubuloglomerular feedback (TGF) in the absence of differences in ECF volume or of alterations in TGF sensitivity during metabolic alkalosis. Of the proposed signals for TGF sensed by the macula densa, distal tubule fluid osmolality or some related variable is the signal most compatible with our data.

Introduction

Renal bicarbonate retention in chloride-depletion alkalosis (CDA)[1] may be facilitated by a reduction in filtered bicarbonate load, an increase in bicarbonate reabsorption, or both. Apparent reductions in glomerular filtration rate (GFR) in CDA have been recognized for many years (1–3). Recently, Cogan and Liu (4) and Maddox and Gennari (5) have provided evidence that reduced GFR—attributed to volume depletion—and not enhanced absolute bicarbonate reabsorption in the superficial proximal convoluted tubule maintains alkalosis. However, other investigators considered that the degree of reduction in GFR in CDA was either out of proportion to (6) or unassociated with demonstrable extracellular fluid (ECF) volume depletion in humans (7) and rats (8).

We have used a model of CDA produced by peritoneal dialysis to distinguish the pathogenetic role of chloride depletion

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1. *Abbreviations used in this paper:* BW(s), body weight; CDA, chloride-depletion alkalosis; CON, control rats; df, degrees of freedom; ECF, extracellular fluid; GFR, glomerular filtration rate; SFP, stop flow pressure; SNGFR(s), superficial nephron glomerular filtration rate; TF_{Cl}, tubule fluid chloride concentration; TF_{osm}, tubule fluid osmolality; TGF, tubuloglomerular feedback.

from that of volume depletion in the maintenance of CDA (9). With this model, we corrected CDA by infusion of fluids containing chloride and bicarbonate in the same concentration as in the plasma of CDA rats but without ECF volume expansion. As compared to controls, we observed a reduction in GFR in CDA rats that was not explained by ECF volume contraction and was corrected by chloride repletion without an increase in plasma volume. Preliminary data suggested a reduction in GFR on the basis of tubuloglomerular feedback (TGF).

The purposes of the present study were to determine whether, independent of alterations in ECF volume, TGF could explain reductions in GFR during CDA and, if so, the nature of the signal perceived by the macula densa. To achieve these goals, we studied three degrees of increasing severity of CDA produced in rats by peritoneal dialysis and examined these with free-flow micropuncture techniques.

Methods

Three degrees of severity of CDA (CDA-1, CDA-2, and CDA-3) were produced by peritoneal dialysis and infusions of various electrolyte solutions in 63 male Sprague-Dawley rats (Charles River Breeding Laboratories, Cambridge, MA) (Table I). The body weights (BW) of the four groups did not differ (control rats [CON] 284±6 g, n = 10; CDA-1 272±9, n = 7; CDA-2 273±10, n = 8; CDA-3 257±17, n = 6; P = NS). All rats ate regular rat chow (Ralston Purina Co., St. Louis, MO) and drank tap water ad libitum until the day of the experiment. They were anesthetized with Inactin (Promonta, Hamburg, Federal Republic of Germany) 100 mg/kg BW and placed on a servo-controlled heated table which maintained rectal temperatures at 37°C. The control rats (group CON) were dialyzed against Ringers-bicarbonate plus glucose 15 gm/L as previously described (10). In the experimental groups (CDA-1, CDA-2, and CDA-3), CDA was produced by dialysate composed of Na-150, K-4, HCO_3-154 meq/L, and glucose (15 gm/L). Group CDA-3 was subjected to two consecutive 30-min dialyses; contemporaneously studied group CON rats were also subjected to two dialyses. Chloride balance for dialysis in group CDA-3 (−3349±215 μeq) was about twice that for a single dialysis, whereas chloride balance for the two exchange group CON (608±181 μeq) was comparable to single exchange group CON rats (10). Because the results in these group CON rats did not differ from single exchange group CON rats with regard to any variable examined, single and double exchange group CON rats are considered as a single group. If arterial blood pressure decreased to 100 mmHg or less in a group CDA-3 rat during the second exchange, the dialysate was removed at that point and the protocol continued. Such decreases were noted in three of six rats, but blood pressures returned promptly to control levels when dialysis was discontinued.

Table I. Major Protocol Variables for the Groups

Group	Dialysate	Infusate
CON	Ringers-HCO_3	Ringers-HCO_3
CDA-1	0.15 M $NaHCO_3$	0.15 M NaCl
CDA-2	0.15 M $NaHCO_3$	0.15 M $NaHCO_3$
CDA-3	0.15 M $NaHCO_3$*	0.15 M $NaHCO_3$

* Two exchanges.

Dialysis-related fluid shifts which were estimated by the change in arterial hematocrit were corrected by 6% bovine serum albumin infusions as previously described (9). All blood samples from the experimental rats were replaced with 45% erythrocytes that had been obtained from littermates and resuspended in a modified Krebs-Ringer bicarbonate solution. Animals were prepared surgically for micropuncture; surgical losses of fluid were not replaced to avoid volume expansion.

Group CON was infused with Ringer-bicarbonate solution, group CDA-1 with 0.15M NaCl, and groups CDA-2 and CDA-3 with 0.15M $NaHCO_3$. Methoxy-^{3}H-inulin (250 μCi/h) was added to all infusates and all animals were infused at 0.5 ml/h per 100 g BW. After 1 h of equilibration, a 1-h clearance period was begun during which micropuncture was carried out.

The procedures for the selection of tubule puncture sites and micropuncture were a modification of those described by Ploth et al. (11). A single 0.05-ml bolus of 5% Food, Drug, and Cosmetic (FD and C) Green 3 (Keystone Aniline & Chemical Co., Chicago, IL) was injected intravenously and the transit time to the late proximal tubule determined. If this transit time was >15.0 s, the animal was discarded. A sharpened pipette with a tip diameter of 2–3 microns was then introduced into a proximal tubule at random and the latest proximal and earliest distal surface convolutions for that nephron were identified with small injections of 5% FD and C Green. A block of water-equilibrated Sudan black-stained mineral oil was introduced at the distal site and a timed collection obtained for determination of volume, osmolality, and inulin and chloride concentrations. Immediately thereafter, an untimed sample of tubule fluid was obtained from the proximal site for determination of inulin and chloride concentrations. 1–4 such paired samples were obtained in each rat. In separate latest proximal tubules that were blocked with oil, 1–4 timed samples were obtained for volume and inulin concentration only. Arterial blood and urine were sampled periodically for determination of osmolality and inulin and chloride concentration. Upon completion of the 1-h clearance period, the animals were killed by aortic puncture and the arterial blood was analyzed for sodium, potassium, chloride, total CO_2 (tCO_2), pH, $PaCO_2$, osmolality, and total protein.

The sensitivity of TGF was studied in separate groups of group CON (n = 4) and CDA-2 (n = 6) animals. The BWs were 307±11 and 304±8, respectively (P = NS). The animals were prepared in a manner identical to that already described except that Ringer-bicarbonate solution was placed in the agar well over the kidney in group CON and 0.15 M $NaHCO_3$ in group CDA-2.

The TGF mechanism was investigated by the response of proximal tubule luminal stop flow pressure (SFP) to changes in orthograde perfusion rate. A 3-μm micropipette connected to a servo-null micropressure system (World Precision Instrument Co., New Haven, CT) was inserted into an early proximal segment for constant recording of proximal tubule pressures. A pipette filled with bone wax was inserted into an intermediate proximal segment and a small cast of wax 4–5 tubule diameters in length was injected into the tubule with a Wells microdrive unit (Trent Wells, South Gate, CA). A pipette filled with artificial tubule fluid which contained 130 mM NaCl, 5 mM KCl, 1 mM $MgSO_4$, 10 mM $NaHCO_3$, 1 mM Na_2HPO_4, 1 mM $CaCl_2$, 4.5 mM urea, and tinted with FD and C Green 3 was then inserted into a late surface proximal segment of the nephron. This pipette was

attached to a microperfusion pump (World Precision Instrument Co.), which had previously been calibrated in vitro. Perfusion of the loop segment was then set at 15, 25, and 40 nl/min and the change in SFP was recorded. Generally, 2–5 min were required for stabilization of the SFP at the new rate. Return to base-line SFP was a consistent finding when the pump was turned off and tubule fluid flow rate fell to zero. 1–4 nephrons were examined in each animal. Upon completion of the experiment, the animals were killed by aortic puncture and the arterial blood was analyzed for chloride, tCO_2, total protein, and hematocrit.

Separate groups of CON and CDA-2 rats (n = 10) and doubly dialyzed CON and CDA-3 rats (n = 6) were prepared in pairs as in the preceding protocols and plasma volumes were determined in pairs by ^{125}I-albumin at the midpoint of the clearance period.

Analytical techniques. The volume of tubule fluid samples was measured in constant-bore glass tubing with a microslide comparator (Gaertner Scientific Corp., Chicago, IL). Tubule fluid chloride concentration was determined by the second method of Ramsay, Brown, and Croghan (12). Tubule fluid osmolality was determined by freezing point depression (Clifton Technical Physics nanoliter osmometer, Hartford, NY). Activities of ^{3}H-inulin in tubule fluid, plasma, and urine were determined by liquid scintillation counting (model 3325 Tri-Carb spectrometer, Packard Instrument Co., Downers Grove, IL).

Sodium and potassium concentrations in urine and plasma were determined by flame photometry (model 243, Instrumentation Laboratory, Lexington, MA) and chloride concentration by electrometric titration (Buchler Instruments, Fort Lee, NJ). Total CO_2 concentration in plasma and urine was determined manometrically (Harleco CO_2 apparatus, American Dade, Miami, FL). Arterial pH and $PaCO_2$ were determined with a BMS3 MK2 blood gas analyzer (Radiometer A/S, Copenhagen, Denmark). Protein concentration was determined by refractometry (model 10400A, American Optical Corp., Buffalo, NY). Plasma volume with ^{125}I-bovine serum albumin was estimated by the method of Belcher and Harriss (13) with a correction for plasma trapping. Plasma osmolality was determined by vapor pressure osmometry (Wescor model 5130C osmometer, Wescor Inc., Logan, UT).

Calculations. Whole kidney GFR and urinary excretions of sodium, potassium, and chloride were calculated according to standard expressions. Ultrafiltrate chloride concentration was obtained by multiplying plasma chloride concentration by the correction factor for plasma water (1.06) as previously suggested (14, 15). The various single nephron functional characteristics, including superficial nephron glomerular filtration rate (SNGFR), absolute and fractional reabsorptions and deliveries of fluid and chloride from both proximal and distal puncture sites, and estimated reabsorptions of fluid and chloride in the loop segment were calculated as previously described (16). Distally determined SNGFR's were used to calculate load to the loop segment. The term "loop segment" refers to the entire nephron segment between the latest proximal and earliest distal surface convolutions. Values given are mean±SEM. Statistical significance among or between groups was assessed by analysis of variance, except that the unpaired "t" test was used between the groups that were studied simultaneously for ^{125}I-albumin determinations of plasma volume. Linear regression analysis was performed by the least squares method (17). Significance was set at the 5% level.

Results

Increasing hypochloremia and hyperbicarbonatemia progressing from group CON to CDA-3 was achieved (Table II). The arterial pH increased progressively to a maximum of 7.68. The plasma sodium concentrations and osmolalities did not differ among the groups.

Comparable volume status for all groups was an important objective of this protocol. Mean arterial blood pressures and hematocrits and plasma protein concentrations did not differ among the groups (Table III). Plasma volumes determined in pairs in groups CON and CDA-2 and groups CON (two exchanges) and CDA-3 also did not differ either between or among groups (P = NS).

Inulin clearance in the experimental kidney decreased together with the plasma chloride concentration but did not decline further in group CDA-3 (Table IV). Urine flow rate was higher in group CDA-3 than that in the other three groups, among which it did not differ. Sodium excretion increased as plasma chloride concentration decreased but potassium excretion was unchanged. Chloride excretions did not differ (P = NS) among all hypochloremic groups (CDA-1, 2, and 3) but were lower than that in controls ($P < 0.01$). Fractional excretion rates for sodium, potassium, and chloride showed similar patterns.

SNGFRs determined from earliest distal puncture sites decreased progressively ($P < 0.001$) from 40.9±1.7 to 28.3±1.8

Table II. Arterial Blood Composition

Group	Na^+	K^+	Cl^-	tCO_2	Osm	pH	$PaCO_2$
	meq·L^{-1}	*meq·L^{-1}*	*meq·L^{-1}*	*meq·L^{-1}*	*mosmol·kg^{-1}*		*mmHg*
CON	141±2	4.3±0.2	101±1	25±1	298±1	7.50±0.01	29±2
CDA-1	141±3	3.5±0.1*	89±2*	32±1*	297±3	7.52±0.03	33±2
CDA-2	140±1	4.1±0.2‡	78±1*‡	37±1*‡	298±3	7.68±0.05*‡	28±1
CDA-3	140±1	3.3±0.2*§	71±1*‡§	43±1*‡§	294±2	7.67±0.03*‡	39±4*§
F	1.12	7.13	85.2	59.2	0.23	8.0	3.57
P value	NS	<0.01	<0.01	<0.01	NS	<0.01	<0.05

* $P < 0.05$ compared to CON. ‡ $P < 0.05$ compared to CDA-1. § $P < 0.05$ compared to CDA-2. NS, not significant.

Table III. Estimates of Fluid Volume Status

Group	BP	Hematocrit	Plasma protein	Plasma volume
	mmHg	%	*gm·dl⁻¹*	*ml·100 g BW⁻¹*
CON	118±3	43.7±0.6	5.0±0.2	(a) 3.86±0.15 (10)* (b) 4.04±0.09 (6)
CDA 1	114±1	45.9±0.9	5.1±0.2	
CDA 2	114±3	46.4±1.2	4.8±0.1	(a) 3.90±0.14 (10)
CDA 3	114±3	44.9±0.7	4.7±0.2	(b) 4.09±0.07 (6)
F	0.6	1.68	0.73	(a) 0.02‡ (b) 0.36‡
P value	NS	NS	NS	NS NS

(a), Single exchange dialysis CON and CDA 2. (b), Double exchange dialysis CON and CDA 3. * Number of observations. ‡ Unpaired "t" value. NS, not significant.

Table V. Superficial Nephron Glomerular Filtration Rates

Group	Determined	
	Distally	Proximally
	nl·min⁻¹	*nl·min⁻¹*
CON	40.9±1.7 (28)	45.2±3.8 (6)
CDA-1	34.4±1.8 (20)*	42.8±2.3 (17)
CDA-2	31.6±1.9 (17)*‡	44.3±3.2 (17)
CDA-3	28.3±1.8 (10)*‡	46.4±5.0 (9)
F	8.30	0.20
P value	<0.01	NS

Number of observations in parentheses.
* $P < 0.05$ compared to CON.
‡ $P < 0.05$ compared to CDA-1.

nl/min with increasing hypochloremia (Table V). The correlation between these SNGFRs and plasma chloride ($r = 0.40$, degrees of freedom (df) = 75) and tCO_2 ($r = -0.40$, df = 75) concentrations were significant ($P < 0.001$). In contrast, SNGFRs determined from latest proximal puncture sites did not differ (P = NS) among the groups and were higher ($P < 0.05$) than distally determined SNGFRs within all CDA groups.

In the loop segment, fractional fluid reabsorption increased as SNGFR and absolute fluid delivery decreased (Table VI). Fractional chloride reabsorption increased and absolute chloride reabsorption decreased with progressive hypochloremic alkalosis. We attribute the progressive decreases in absolute chloride reabsorption in the loop segment to the progressive decreases in delivery (18) as well as an effect of hypochloremia independent of delivery (19).

Tubule fluid flow rates in the early distal convolution decreased progressively as SNGFR decreased (Table VII). Early distal tubule fluid chloride concentration (TF_{Cl}) also decreased in this manner and correlated positively with distally determined SNGFR ($r = 0.35$; df = 75; $P < 0.01$). Tubule fluid-to-ultrafiltrate chloride ratios did not differ (P = NS) among the CDA groups. In contrast, early distal TF_{osm} and tubule fluid-to-plasma osmolality ratios both increased as SNGFR decreased. Early distal TF_{osm} correlated negatively with distally determined SNGFR ($r = -0.32$; df = 58; $P < 0.05$).

TGF sensitivity was examined in groups CON and CDA-2 in which blood pressure (114±4 and 108±3 mmHg), plasma protein concentration (4.9±0.2 and 5.1±0.1 g/dl), and arterial hematocrit (43.2±1.5 and 44.0±0.4%), respectively, did not differ (P = NS). In group CDA-2, plasma chloride was lower (87±4 vs. 109±2 meq/L; $P < 0.005$) and tCO_2 higher (36±1 vs. 25±1 meq/L; $P < 0.005$) than those in group CON. SFP did not differ (P = NS) between groups CON (40±1 mmHg) and CDA-2 (38±2). The sensitivity of SFP to increasing rates of orthograde perfusion of the loop segment also did not differ between groups (Fig. 1).

Discussion

We produced three grades of severity of hypochloremic metabolic alkalosis in this study. In association with progressive

Table IV. Experimental Kidney Functions

Group	Inulin clearance	Urine flow	Sodium excretion		Potassium excretion		Chloride excretion	
	μl·min⁻¹	*μl·min⁻¹*	*neq·min⁻¹*	% of filtered	*neq·min⁻¹*	% of filtered	*neq·min⁻¹*	% of filtered
CON	1,667±142	7.5±1.6	529±206	0.26±.08	1,697±196	25.4±3.1	708±176	0.44±0.09
CDA-1	1,402±135	8.9±2.7	926±342	0.42±.13	1,706±189	37.6±3.9	46±14*	0.04±0.01*
CDA-2	1,207±55*	9.8±1.6	2,140±484*‡	1.20±.23*‡	2,010±223	39.8±5.4	33±6*	0.04±0.01*
CDA-3	1,291±80*	16.8±2.5*‡	3,459±1088*‡	1.62±.37*‡	1,674±264	39.5±9.8	59±13*	0.06±0.02*
F	3.29	3.61	6.50	9.80	0.59	2.07	9.59	11.44
P value	<0.05	<0.05	<0.01	<0.01	NS	NS	<0.01	<0.01

* $P < 0.05$ compared to control. ‡ $P < 0.05$ compared to CDA-1.

Table VI. Loop Segment Fluid and Chloride Reabsorption

Group	Fluid		Chloride	
	nl·min⁻¹	%	*peq·min⁻¹*	%
CON	10.4±1.0	55.1±3.3	2,009±122	86.3±1.2
CDA-1	10.4±2.5	57.3±2.9	1,689±132	89.8±1.1*
CDA-2	12.2±1.2	70.7±2.7*‡	1,492±147*	92.2±1.8*
CDA-3	7.1±1.2§	72.9±4.7*‡	765±128*‡	93.8±1.1*‡
F	2.22	6.68	10.36	5.45
P value	NS	<0.01	<0.01	<0.01

* $P < 0.05$ compared to CON. ‡ $P < 0.05$ compared to CDA-1. § $P < 0.05$ compared to CDA-2. NS, not significant.

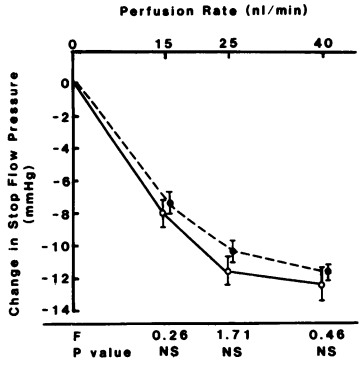

Figure 1. Relationship of absolute changes in proximal tubule SFP to orthograde perfusion rate of the loop segment in groups CON (○) and CDA-2 (●). Statistical notation is given for each perfusion rate.

hypochloremic alkalosis, SNGFRs determined from distal puncture sites decreased progressively, whereas SNGFRs determined from proximal sites did not change. In addition, as CDA increased in severity, early distal TF_{Cl} and flow rate and chloride reabsorption in the loop segment decreased progressively, whereas early distal TF_{osm} inreased.

Occult volume contraction seems a highly unlikely explanation for the decreases in distally determined SNGFR. Based on determinations of blood pressure, arterial hematocrit, plasma protein concentration and ^{125}I-albumin space, we could detect no differences in ECF volume at the time of study. Because mean arterial pressure and plasma protein concentration as well as proximally determined SNGFRs did not differ among the groups, the systemic hemodynamic determinants of SNGFR do not appear to explain our findings (20). Thus, we conclude that SNGFR in this model of metabolic alkalosis was reduced by stimulation of TGF and not by volume contraction.

The sensitivity of TGF increases when volume contraction is overt. Moore et al. (21) showed that acute changes in fluid volume produced a rapid increase in the sensitivity of TGF. In their study, SNGFR was estimated by early proximal tubule fluid flow rate, thus precluding direct comparisons with our data. However, when increased sensitivity of TGF was demonstrated, BW was decreased 2.2±0.2%, blood pressure was decreased 9.0±2.0 mmHg, and hematocrit increased 0.03±0.002 vol %, all of which were significant changes. They estimated the decrease in ECF volume to be 9%. None of these variables that were examined in our experiments showed such differences. Kaufman et al. (22) studied TGF after hemorrhage or aortic constriction by observation of the responses of proximally and distally determined SNGFRs. They were unable to show an increase in the sensitivity of TGF unless they produced severe hemorrhagic hypotension—mean arterial pressure of 70±1 mmHg. Likewise, Selen and Persson (23) showed a resetting of TGF sensitivity during reduction in renal arterial pressure from 115 to 75 mmHg. Selen et al. (24) also showed activation of TGF by dehydration but made no assessment of the magnitude of ECF volume contraction. Based on the observations of these investigators and our assessment—by at least as precise estimates—that the ECF volume among the four

Table VII. Early Distal Tubule Fluid Characteristics

Group	Flow rate	TF_{Cl}	TF/UF_{Cl}	TF osm	TF/P osm
	nl·min⁻¹	*meq·L⁻¹*	*mosmol·L⁻¹*	*mosmol·L⁻¹*	*mosmol·L⁻¹*
CON	8.6±0.7 (24)	35.5±1.7	0.34±0.02	110±5	0.38±0.02
CDA-1	7.3±0.5 (21)	25.5±1.5*	0.27±0.02*	142±6*	0.47±0.02*
CDA-2	5.5±0.7*‡ (21)	24.7±2.8*	0.29±0.04	187±10*‡	0.63±0.03*‡
CDA-3	3.4±0.6*‡§ (15)	18.9±2.6*‡	0.28±0.03	208±12*‡	0.71±0.04*‡
F	10.67	9.74	1.54	30.41	19.9
P value	<0.01	<0.01	NS	<0.01	<0.01

Number of observations in parentheses. * $P < 0.05$ compared to CON. ‡ $P < 0.05$ compared to CDA-1. § $P < 0.05$ compared to CDA-2.

groups in present study did not differ, we suggest that SNGFR was not indirectly reduced by an increase in TGF sensitivity that was produced by volume contraction. Furthermore, alkalosis at an intensity associated in the present study with a reduced SNGFR did not influence significantly the sensitivity of TGF to changes in orthograde perfusion rate of the loop segment, a procedure which is commonly employed to test this characteristic (21, 23). However, even if a change in TGF sensitivity did occur, enhancement by acid-base changes would not be inimical to our interpretation that the observed differences in SNGFR and GFR are not dependent on ECF volume depletion.

Several putative signals for the afferent limb of TGF—decreasing SNGFR in response to increasing tubule fluid flow rate, sodium or chloride concentration, or osmolality, or chloride reabsorptive rate in the ascending limb of the loop of Henle—have been suggested (25–28), and the nature of this signal continues to be vigorously debated (29). We have examined all of these proposed signals except sodium in this study. Our data show that, as SNGFR decreases, early distal TF_{Cl}, tubule fluid flow rate, and absolute chloride reabsorption in the loop segment all decrease progressively. An increase in luminal chloride concentration between macula densa and earliest surface convolutions has been described (30) and is one potential source of error for our study. If osmotic water flux produces this increase, as proposed by Gutscher et al. (31), the progressive decrease in the transepithelial solute gradient with increasing severity of CDA would only serve to diminish the observed difference in TF_{Cl} among the groups. Alternatively, Schnermann et al. (30) have proposed that this increase in luminal chloride is produced by chloride secretion, which is enhanced by decreasing flow rate or decreasing NaCl concentration in tubule fluid. If this explanation is correct, we would have progressively and systematically overestimated rather than underestimated chloride concentrations at the macula densa with increasing CDA. Furthermore, the unchanging transepithelial chloride gradient among the CDA groups would not have contributed any systematic error.

Thus, notwithstanding these uncertainties, TF_{Cl} and flow rate and absolute loop segment chloride reabsorption in the present study changed in direction opposite to that proposed from studies which examined these signals for TGF by microperfusion techniques. In contrast, we showed that, as distal TF_{osm} increased progressively from 110 to 208 mosmol $\cdot L^{-1}$, SNGFR decreased progressively from 40.9 to 28.3 nl/min, which was consistent with the directional change suggested by Bell and Navar (28). During retrograde microperfusion of the loop segment, they showed a progressive decrease in SFP to a maximum of −12 mmHg as distal osmolality was increased from 38 to ~125 mosmol $\cdot L^{-1}$. The difference in the magnitude between our data and those of Bell and Navar (28) may be due to the different techniques used. Thus, our data obtained with free-flow micropuncture techniques and in the presence of a systemic stimulus to TGF are consistent with the proposal of Bell and Navar (28) that TF_{osm} is the signal perceived by the macula densa. Since it is likely that the increase in early distal TF_{osm} is due mainly to bicarbonate and obligated sodium, the presumed increase in the concentration of either of these ions may conceivably also serve as the signal perceived by the macula densa. Finally, we cannot definitively exclude an effect of impermeant anions—such as bicarbonate—in the lumen to enhance the efficacy of chloride as the signal as proposed by Schnermann and Briggs (32).

Although SNGFR declined progressively with increasing CDA, the differences between some adjacent groups, e.g., CDA-3 vs. CDA-2, were not significant. Likewise, experimental kidney GFR reached a minimum in group CDA-2 and did not decrease further in group CDA-3. We do not know whether similar changes in SNGFR in or solute delivery to the macula densa of juxtamedullary nephrons occurred in response to CDA; indeed, little is known of TGF in juxtamedullary nephrons (33). In addition, if the reduction in SNGFR that we observed approaches the maximum attainable through TGF (27), it may be more difficult to show differences in whole kidney GFR as CDA intensifies.

Potassium balance in these groups did not differ because neither the infusion nor the excretion rates of potassium differed. Hypokalemia, probably due to intercompartmental shifts, was inconstant in this study, and, thus, is unlikely to have systematically influenced the results. Moreover, acute hypokalemia, as distinct from potassium depletion, has not been shown to alter proximal tubule bicarbonate reabsorption (34, 35).

How could TGF-mediated reductions in GFR fit into the pathophysiology of metabolic alkalosis? Early in the course of CDA—during the disequilibrium phase—TGF stimulation would serve to prevent volume contraction by limiting the delivery of bicarbonate to the distal nephron, and thereby blunting any obligatory loss of sodium and fluid. Such an effect is illustrated by comparison of filtered bicarbonate load calculated from either distally determined or proximally determined SNGFRs (Table VIII). The prevention of bicarbonate overload is clearly seen. In this schema, if volume contraction supervenes, additional mechanisms to reduce GFR further and to enhance fluid reabsorption serve to intensify the conservation

Table VIII. Comparison of Filtered Bicarbonate Load

Group	Distal*	Proximal‡	Difference
	pmol · min⁻¹	*pmol · min⁻¹*	*pmol · min⁻¹*
CON	1,022	1,130	108
CDA-1	1,101	1,370	269
CDA-2	1,169	1,639	470
CDA-3	1,217	1,995	778

* Calculation based on distally determined SNGFR.
‡ Calculation based on proximally determined SNGFR.

of fluid, sodium, and chloride. Thus, TGF is a mechanism both to prevent urinary sodium and bicarbonate loss and, hence, volume contraction, and, when volume contraction is also present, to supplement fluid and sodium conservation.

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