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ORIGINAL ARTICLE

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An analysis of renal tubular acidosis by the Stewart method

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Abstract Renal tubular acidosis (RTA) comprises a group of disorders characterized by a low capacity for net acid excretion and persistent hyperchloremic, metabolic acidosis. To investigate the role of chloride, we performed hypotonic (0.45%) saline-loading experiments in 12 children with alkali-treated distal RTA (dRTA) and compared the results with data obtained from 17 healthy control subjects. In patients, but not in controls, saline loading induced both hyperchloremia and metabolic acidosis. Hyperchloremia was associated with high total and high distal fractional reabsorption of chloride [$C_{H20}/(C_{H20}+C_{Cl})$]. The increase in plasma chloride varied inversely with the fractional excretion of chloride (C_{Cl}) and correlated with the decrease in blood pH. However, the urinary excretion of bicarbonate did not correlate with either changes in blood pH or plasma bicarbonate concentration. Our findings suggest that the mechanism of hyperchloremia was enhanced Cl^-/HCO_3^- exchange by the distal tubule. The resulting metabolic acidosis is better explained by changes in the strong ion difference (the Stewart theory) than by changes in the urine bicarbonate excretion (the traditional theory).

Keywords Strong ion difference · Renal tubular acidosis · Stewart theory · Acid–base balance · Hyperchloremia · Cl^-/HCO_3^- exchange

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Introduction

Renal tubular acidosis (RTA) comprises a group of disorders that are characterized by the inability of the kidneys to excrete an acid load as a result of defects or mutations in renal tubular H^+ and HCO_3^- transport proteins [1, 2]. The long-accepted, traditional model of acid–base balance supposes that plasma [H^+] is determined by the balance between hydrogen ion generation and urinary hydrogen ion secretion. According to this view, RTA, *primarily* due to a defect in hydrogen ion transport proteins, results *secondarily* in a positive hydrogen ion balance.

Recently, Stewart, a Canadian physiologist, proposed a new theory of acid–base balance based principally upon the laws of conservation of mass and charge [3]. Assuming that human plasma consists of fully dissociated ions (strong ions, such as Na^+, K^+, Cl^-, lactate), partially dissociated weak acids (such as albumin and phosphate), and volatile buffers (carbonate species), he derived a fourth-order polynomial equation that related [H^+] to three independent variables, the strong ion difference [SID], the total concentration of weak acids [A_{TOT}], and P_{CO2} [3, 4, 5] According to this view, renal tubular acidosis is due *primarily* to a defect in hydrogen ion transport proteins, resulting *secondarily* in "mishandling" of chloride ions. It is precisely this "mishandling" of chloride that causes a low [SID], resulting in acidosis.

To investigate the validity of Stewart hypothesis in this population, we performed hypotonic (0.45%) saline-loading experiments on patients with alkali-treated distal RTA (dRTA) and compared the results with a second group of healthy control subjects. As expected, saline loading did not disturb acid–base balance in healthy subjects. However, saline loading resulted in significant hyperchloremia and acidosis in patients with dRTA. Clearance studies revealed that hyperchloremia was due to high tubular reabsorption of chloride and was proportional to the degree of acidosis.

Table 1 Plasma values (mean ± SD) before and after hypotonic (0.45%) saline infusion (2 l/1.73 m^2 over 120 min). Normal laboratory reference range values are given in reference [11] and normal post-saline clearance values are given in reference [10]

Parameter	Reference range (n=15)	Distal RTA (n=12)		Normal (n=17)
		Before saline infusion	After saline infusion	After saline infusion
pH	–	7.41±0.06	7.38±0.06[a]	
HCO_3^- (mM)	22.5±2.8	21.8±4.2	18.6±3.4[a,c]	22.5±1.2[b]
TCO_2 (mM)	–	22.8±4.3	19.2±3.7[a]	
P_{CO2} (Torr)	–	36.0±6.5	34.4±7.6[a]	
Na (mM)	141.3±3.9	138.2±3.5[c]	139.1±3.3	139.9±3.2
Cl (mM)	106.1±3.9	106.7±3.6	111.0±4.9[a]	105.6±7.8[b]
K (mM)	4.5±0.38	3.8±0.6[c]	3.5±0.5[a,c]	3.8±0.3[c]

[a] P<0.05 vs pre-saline values of dRTA patients, paired t-test
[b] P<0.05 vs post-saline values of dRTA patients, signed rank sum test and unpaired t-test
[c] P<0.05 vs normal reference range, unpaired t-test

Subjects and methods

Subjects

Twelve unrelated patients (nine male, three female) with primary dRTA were studied at the mean age of 4.5 years (range 0.5 to 12.5 years). The diagnosis of dRTA was made during infancy or early childhood because of the presence of growth failure, polyuria and nephrocalcinosis. The children all exhibited a persistent inability to lower urinary pH during ammonium chloride-induced metabolic acidosis. Molecular biology studies have been reported in 11 patients. One patient was affected by the autosomal dominant form of dRTA and presented a de novo mutation in the *SLC4A1* gene, encoding the AE1 anion exchanger [6]; two autosomal recessive cases, associated with early neurosensory deafness, presented mutations in the *ATP6V1B1* gene, encoding the B1 subunit of H^+ ATPase [7]; and eight autosomal recessive cases, with or without late neurosensory deafness, presented mutations in the *ATP6V0A4* gene, encoding the a4 subunit of H^+ ATPase [8].

The control group was formed of 17 healthy children, aged 2 years to 12 years, previously studied by the same methodology [9, 10].

In every case the parents were informed of the studies, and signed consent was obtained.

Methods

Clearance studies during hypotonic saline diuresis

The protocol used has been already reported [9, 10]. All patients continued their usual alkali therapy. On the morning of the study each subject received an oral water load of 20 ml/kg body weight over a 30-min period. This was followed by the intravenous infusion of 0.45% saline solution at a rate of 1,000 ml/h per 1.73 m^2 of body surface, over a period of 2 h. Patients and control children were not catheterized, since the results for each period were factored by glomerular filtration rate (GFR) so that accurately timed urine collections were, in consequence, not required. Specimens of urine were obtained at 20-min intervals, and blood samples were drawn at the midpoint of each urine collection from an indwelling needle that was flushed with slightly heparinized isotonic saline solution after each sampling. Acid–base variables were determined in capillary arteriolized blood.

Laboratory determinations

All clearance values were expressed as milliliters per deciliter GFR. The period with maximal free water clearance was used for the analysis. Osmolality and electrolytes were measured by standard laboratory methods. Blood pH and P_{CO2} were measured with the Astrup apparatus. Assays for plasma urine creatinine were performed with a Beckman autoanalyzer, by modification of the Jaffe method.

Calculation of [SID]

[SID] was calculated from $[SID]=[Na^+]+[K^+]-[Cl^-]$ [3]

Statistics

The calculation of simple linear regression, multiple regression, the signed rank sum non-parametric test, paired t-test and unpaired t-test was performed with MedCalc Statistical Software, version 8.0, Mariakerke, Belgium.

Results

Plasma electrolytes

In the controls, saline loading resulted in no significant changes in electrolyte or acid–base balance (Table 1). In contrast, most children with treated dRTA who were given a saline load developed hyperchloremia, hypobicarbonatemia, hypocarbia and acidemia. However, one patient (affected by a mutation in the AE1 anion exchanger) presented with a decrease in plasma chloride and no evident changes in blood pH or plasma bicarbonate concentration (Fig. 1, Table 1).

Clearance studies: sodium and chloride

Data are presented in Table 2. In healthy controls, C_{Cl} was greater than C_{Na}, and the fractional distal reabsorption of sodium $[C_{H20}/(C_{H20}+C_{Na})]$ was also greater than the fractional distal reabsorption of chloride $[C_{H20}/(C_{H20}+C_{Cl})]$. In children with treated dRTA, C_{Cl} was approximately equal to C_{Na}, and the fractional distal reabsorption of sodium $[C_{H20}/(C_{H20}+C_{Na})]$ was approximately equal to the fractional distal reabsorption of chloride $[C_{H20}/(C_{H20}+C_{Cl})]$. These observations suggest that the comparatively low fraction excretion of chloride might explain the observed increase in plasma chloride.

To explore this possibility, the derived values (Tables 1 and 2) may be used to calculate the filtered load and the amounts of proximal tubular reabsorption, distal delivery, distal tubular reabsorption, and final excretion of chloride and sodium in dRTA patients and in control subjects (Table 3). These calculations reveal that both the

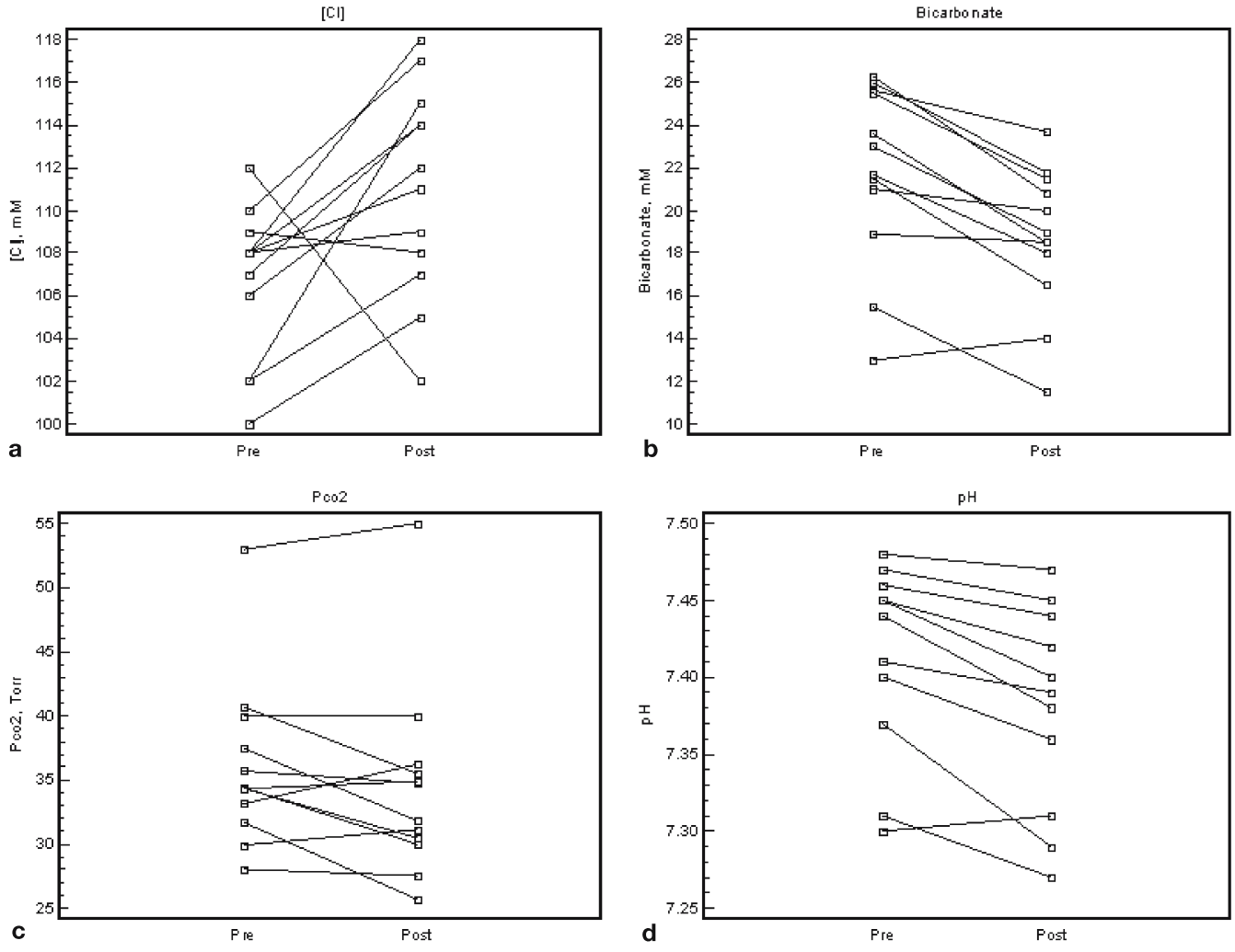

Fig. 1 Values of plasma chloride (mM), bicarbonate (mM), P_{CO2} (Torr) and pH pre- and post-saline infusion in children (n=12) with dRTA (P<0.05 in all panels, paired t-test)

amount of chloride reabsorbed distally and the total reabsorption of chloride were higher in dRTA patients than in controls. Also, the saline-induced changes in plasma chloride correlated inversely with the clearance of chloride (C_{cl}) (Fig. 2).

Clearance studies: bicarbonate

In both patients and controls, all the filtered bicarbonate appeared to be reabsorbed by the proximal tubule, so that delivery of bicarbonate to the distal tubule was almost absent (Table 3). Therefore, all urinary bicarbonate was formed via secretion by the distal tubule. In patients with dRTA, the mean calculated bicarbonate excretion was 0.15±0.03 mM/min per dl GFR, a value that was not significantly different from measured urine bicarbonate but that was different from controls (0.01 mM/min per dl GFR) (P<0.05, signed rank sum test) (Tables 2 and 3). The rate of bicarbonate excretion paralleled the distal reabsorption of chloride (Fig. 3).

Table 2 Clearance studies during hypotonic saline (0.45%) infusion (mean ± SD). Normal values are given in reference [10]. V, C_{Na}, C_{Cl}, C_{H20}, C_K fractional volume and fractional sodium, chloride, water and potassium clearances, respectively, $C_{H20}/(C_{H20}+C_{Na})$ percent of distal sodium reabsorption, $C_{H20}/(C_{H20}+C_{cl})$ percent of distal chloride reabsorption

Parameter	Distal RTA (n=12)	Normal (n=17)
Urine		
V (ml/dl GFR)	20.4±4.4	17.2±2.7
C_{Na} (ml/dl GFR)	2.9±1.3	1.4±0.4[a]
C_{Cl} (ml/dl GFR)	3.0±1.3	2.1±0.7[a,c]
C_K (ml/dl GFR)	20.5±7.4	12.9±5.2[a]
C_{H2O} (ml/dl GFR)	15.6±3.5	14.0±2.6
$C_{H2O}/(C_{H2O}+C_{Na})$ (%)	84.4±4.1	90.9±3.3[a]
$C_{H2O}/(C_{H2O}+C_{Cl})$ (%)	84.2±5.5	86.7±4.1[a,b]
T_{CO2} (mM/min per dl GFR)	0.18±0.13	–
pH	7.17±0.12	–

[a] P<0.05 vs distal RTA, signed rank sum test and unpaired t-test
[b] P<0.05 vs normal [$C_{H2O}/(C_{H2O}+C_{Na})$] (%), unpaired t-test
[c] P<0.05 vs normal C_{Na}, unpaired t-test

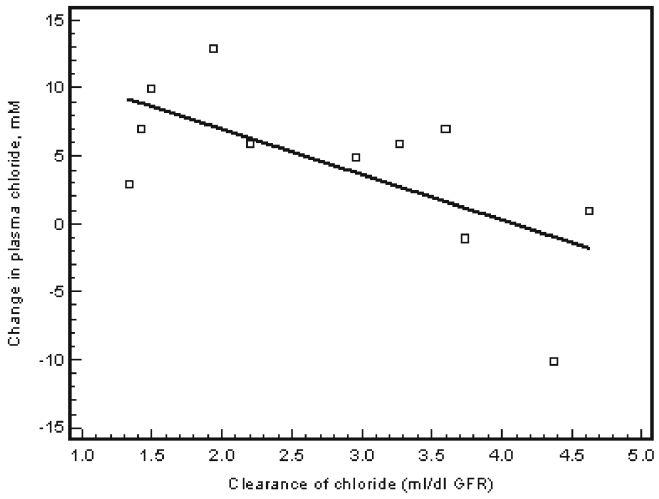

Fig. 2 Correlation between the change in plasma chloride concentration (chloride final−chloride initial, mM) and the fractional clearance of chloride (ml/dl GFR) during hypotonic saline infusion in patients with dRTA (n=11), r=−0.66, P<0.05. One outlier, with GFR <50 ml/min per 1.73 m^2, was omitted

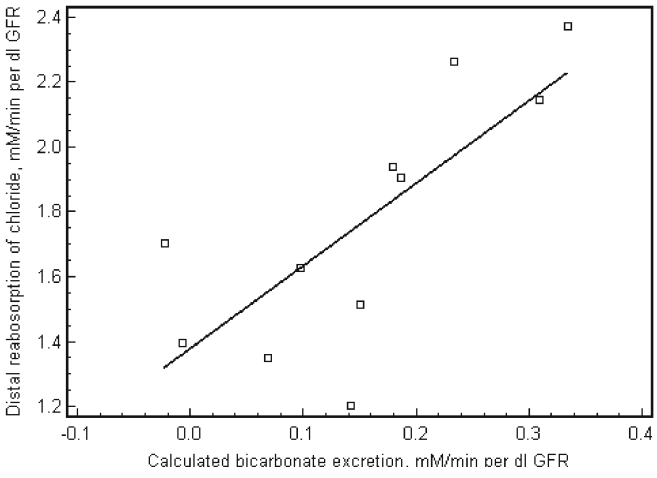

Fig. 3 In patients with dRTA (n=11), plot of calculated bicarbonate excretion (mM/min per dl GFR) vs distal reabsorption of chloride (mM/min per dl GFR), r=0.75, P<0.05, according to the regression Y=1.3905+2.5180X. A similar plot is obtained when measured urinary bicarbonate is used, n=9, r=0.73, P<0.05 (data not shown)

The inter-group difference in mean distal reabsorption of chloride (0.26 mM/min per dl GFR) is the same order of magnitude as the inter-group difference in mean bicarbonate excretion (0.14 mM/min per dl GFR). Charge balance is maintained by the distal reabsorption of sodium (inter-group difference 0.2 mM/min per dl GFR). The y-intercept of the regression line (1.39 mM/min per dl GFR) is similar to the distal reabsorption of chloride in healthy subjects (1.5 mM/min per dl GFR), where distal chloride reabsorption appears to occur via paracellular pathways rather than by an anion-exchange mechanisms.

Strong ion difference

Hyperchloremia was associated with a significant decrease in [SID], from 35.4±1.1 (SEM) mM before saline infusion to 31.6±1.5 (SEM) mM after saline infusion (P<0.05, paired t-test) (Fig. 4).

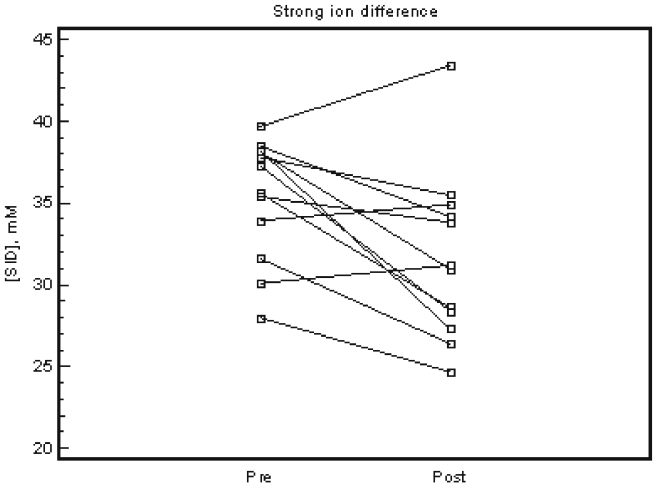

Fig. 4 Strong ion difference [SID] measured pre- and post-saline infusion in children (n=12) with dRTA, P<0.05

Table 3 Tubular reabsorption of electrolytes during hypotonic (0.45%) saline infusion in patients (n=11) with dRTA and in controls (mean ± SEM), mM/min per dl GRF. One patient, with loss-of-function mutation in AE1, developed a decreasing plasma value of Cl after infusion and was excluded. The filtered load is calculated from post-saline infusion values of plasma Cl, Na, K, Cl and T_{CO2}. Normal values are given in parenthesis

Parameter	Cl	Na	K	T_{CO2}
Filtered load	11.18±0.04[b] (10.6)	13.91±0.33[a] (14)	0.35±0.05[a,b] (0.38)	1.98±0.37[a] (2.25)
Proximal reabsorption	9.05±0.74 (8.9)	11.32±0.75[a] (11.8)	–	2.30±0.45[a,b,c] (2.9)
Fractional distal delivery (%)	18.69±0.22 (16.1)	18.76±0.04 (15.4)	–	0
Distal delivery	2.08±0.42[b] (1.7)	2.61±0.60[a] (2.2)	–	0
Distal reabsorption	1.76±0.37[b] (1.5)	2.2±0.50[a] (2.0)	–	–
Total reabsorption	10.86±0.51[b] (10.4)	13.51±0.40[a,b] (13.8)	–	–
Excretion	0.32±0.42[b] (0.21)	0.40±0.18[a,b] (0.2)	0.072±0.03[a,b] (0.02)	0.15±0.12[a,b,d] (0.01)

[a] P<0.05 vs chloride values, paired t-test
[b] P<0.05 vs normal values, signed rank sum test
[c] Calculated as the difference between proximal Na and proximal Cl reabsorption
[d] The urine bicarbonate was calculated as the sum of excreted (Na^++K^+−Cl^-, assuming a low number of urine ammonium ions in dRTA. There is no significant difference in the calculated and measured values of urine bicarbonate (Table 2, P>0.05, paired t-test)

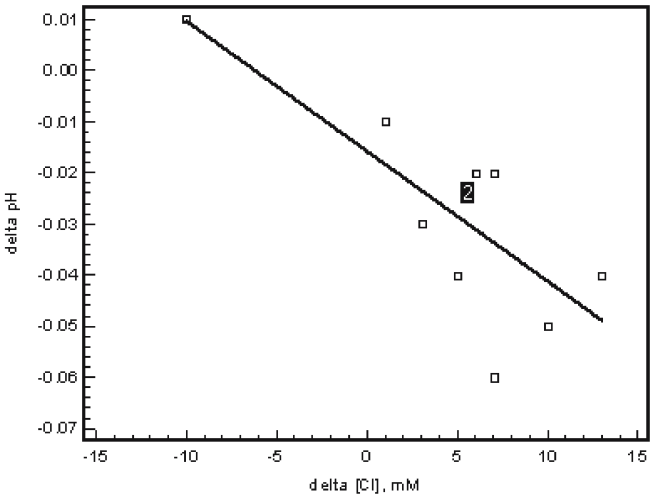

Fig. 5 The correlation between the change (pre- and post-saline infusion) in plasma chloride concentration (delta [Cl]) and the change in pH (delta pH) (r=–0.77, P<0.05, n=10). The symbol *2* indicates two overlapping data points

With the exclusion of two outliers in which the [SID] had increased after saline infusion (Fig. 4), the rise in plasma chloride concentration correlated with the observed decrease in pH (r=–0.77, P<0.05) (Fig. 5). In a forward multiple regression model, the addition of the covariable delta P_{CO2} improved the correlation from r=–0.77 to r=0.88 (P<0.05). On the other hand, the excretion of bicarbonate did not correlate with either the change in blood pH or plasma bicarbonate concentration.

Discussion

Although hyperchloremia is a characteristic feature of dRTA, its pathophysiological role in this disorder remains poorly understood. In the present study, we found that hypotonic saline loading of alkali-treated patients with dRTA was associated with a significant increase in plasma chloride. This observation has not been reported previously and has not been reported in healthy subjects.

This finding may be explained by fractional clearance studies, a method described previously [9, 10]. As contemporaneous data for healthy subjects was not available, we used previously published, historical control data that were obtained by identical methods [10, 11]. We used parametric and non-parametric tests to evaluate the differences between groups, and the accrued data are internally consistent and appear to reveal important differences between dRTA patients and controls.

The total, tubular reabsorption of chloride (mM/min per dl GFR) was higher in patients with dRTA than in healthy controls. The additional retained chloride (on average 0.26 mM/min per dl GFR) varied inversely with the fractional excretion (C_{Cl}) and appears to account for the observed increase in plasma levels. For example, a 10 kg child with dRTA and normal renal function given a hypotonic saline load over 120 min would retain an excess of 31 meq of chloride. This would result in an ~5 meq/l rise in serum concentration, similar to the mean of the increase observed in our patients.

Although the proximal tubule behaved similarly in both groups, the distal tubule of dRTA patients reabsorbed both a higher percentage (16% vs 14%) and a greater absolute amount (1.76 vs 1.5 mM/min per dl GFR) of the filtered chloride load. These findings point to the distal nephron as the major site of excess chloride retention.

The difference in distal fractional chloride reabsorption between groups (0.26 mM/min per dl GFR) was similar to the rate of bicarbonate loss observed in patients with dRTA (0.14 mM/min per dl GFR). Bicarbonaturia was negligible in controls (0.01 mM/min per dl GFR). In patients with dRTA, the rate of distal reabsorption of chloride was proportional to the rate of bicarbonate excretion, maintaining electro-neutrality. The Y-intercept of the regression (1.39 mM/min per dl GFR), at which point bicarbonaturia is absent, was similar in value to the distal chloride reabsorption in controls (1.5 mM/min per dl GFR). These findings suggests that volume-expansion with hypotonic saline unmasks novel distal Cl^-/HCO_3^- exchange in alkali-treated patients with dRTA, accounting for the observed hyperchloremia and metabolic acidosis.

This suggestion is consistent with the proposed cellular mechanism(s) of dRTA. For example, entry and exit of charged species from intercalated cells is governed by families of ion-specific chloride channels, the Na–K–2Cl co-transporter, proton pumps and various Cl^-/HCO_3^- exchangers [12, 13]. The activity and functioning of these transport proteins depends upon several factors, including genetic polymorphism, the ClC-kb co-factor barttin, membrane sorting, cell swelling and intracellular pH. As loss of function mutations result in a hypochloremic, metabolic alkalosis, it is not unreasonable to suppose that upregulation or gain of function mutations of these same proteins may result in a hyperchloremic, metabolic acidosis.

For example, some investigators have proposed that a mutation in the *SLC4A1* gene results in the missorting of a variably-functioning AE1 anion (Cl^-/HCO_3^-) exchanger to the apical surface of intercalated cells [14]. An analogous remodeling of the cell may occur in patients with mutations encoding for subunits of apical H^+ ATPase, in which the intracellular pH of α-intercalated cells (H^+-secreting) may be low. In cell culture, Schwartz et al. [15] found that the intracellular acidosis induces a reversal of cell polarity, so that α-intercalated cells (H^+ secreting) functionally resemble β-intercalated cells (HCO_3^- secreting). In both these models, plasticity of the cell would induce high Cl^-/HCO_3^- exchange across the apical membrane, resulting in both hyperchloremia and metabolic acidosis. The finding that the only patient with a loss-of-function mutation of the AE1 anion (Cl^-/HCO_3^-) exchanger did not develop either hyperchloremia or metabolic acidosis after saline loading strongly favors this hypothesis.

Although an "acid load" was not administered, hypotonic saline infusion was associated with significant metabolic acidosis. The decrease in plasma pH correlated with the increase in plasma chloride but not with the urinary excretion of bicarbonate. The physiological explanation for this is not readily apparent from traditional theories of acid–base balance, based upon the Henderson–Hasselbalch equation. However, newer theories of acid–base balance may provide a solution.

In 1948, Singer and Hasting proposed measuring the difference between plasma cations and anions (buffer base) to determine the corresponding concentration of acids and bases, based upon the principal of electroneutrality [16]. Subsequently, Stewart revised the buffer–base concept by providing mathematical formulae to create a physiochemical model that related a set of independent variables ([SID], $[A_{TOT}]$ and P_{CO2}]) to a set of dependent variables (pH and $[HCO_3^-]$). If non-carbonate buffers are held constant, then, according to this model, metabolic acidosis is due exclusively to a low [SID].

As a corollary, the pH of any fluid compartment is determined exclusively by the independent variables present in that particular compartment. It follows that the kidney maintains acid–base balance mostly through the regulation of plasma $[Na^+]$ and $[Cl^-]$, and thus the [SID]. In contrast to the traditional model, systemic acid–base disturbances may be understood without reference to the urine concentration of ammonium, titratable acid, bicarbonate or hydrogen ions (net acid excretion). While acknowledging the role of hydrogen ion transport proteins in instigating RTA, Stewart claimed that the resulting systemic acidosis could be explained and quantified by the "mishandling" of chloride ions.

The Stewart model may be useful for explaining and quantifying our results. First, the Stewart model explains the mechanism of the saline-induced metabolic acidosis, through an effect on [SID]. Second, the Stewart model permits quantification of the acid–base derangement. For example, we found that plasma chloride increased in proportion to the decrease in the plasma pH ($r=-0.77$, $P<0.05$). In a multiple regression model, the acidosis could be accounted for almost entirely by the changes in $[Cl^-]$ and P_{CO2} (multiple regression $r=0.88$, $P<0.05$).

In summary, our findings suggest that dRTA is often a "plasticytopathy", principally due to aberrant targeting of functional Cl^-/HCO_3^- exchange proteins to the apical surface of intercalated cells. The metabolic acidosis that occurs, following hypotonic saline infusion in alkali-treated patients with dRTA, is better explained by changes in the strong ion difference (the Stewart theory) than by changes in urine bicarbonate excretion (the traditional theory).

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