Renal Acid-Base Excretion in Normotensive Salt-Sensitive Humans

Arya M. Sharma, Claudia Cetto, Ulrike Schorr, Klaus-P. Spies, Armin Distler

Reduced extracellular pH and bicarbonate levels have recently been reported in normotensive salt-sensitive subjects. To assess the possible role of altered renal acid-base handling in the perturbation of acid-base status in these individuals, we measured the renal acid-base excretion after an acute oral administration of either an alkali or acid load in normotensive salt-sensitive and salt-resistant men. Twenty-four young (22 to 29 years old), healthy male volunteers were placed on a low-salt diet (20 mmol NaCl per day) for 2 weeks with either 220 mmol NaCl or placebo added to the low-salt diet for 1 week each in a randomized single-blind crossover order. Salt sensitivity was defined as a significant drop in mean arterial pressure (>3 mm Hg, mean of 60 readings taken on the seventh day of each diet, P<.05) during the low-salt diet. On the fifth and seventh days of each week, subjects were given an oral load of either sodium citrate (0.7 mmol/kg) or ammonium chloride (2.2 mmol/kg), respectively, in a randomized order, and arterial and urinary acid-base status was assessed at baseline and followed for 8 hours thereafter. According to the above definition, 13 subjects were considered salt sensitive. During the high-salt diet, mean arterial pressure was higher in the salt-sensitive than in the salt-resistant group (P<.01). Cumulative urinary bicarbonate excretion after the administration of sodium citrate was lower in the salt-sensitive than in the salt-resistant subjects during both the low-salt (46%, P<.001) and high-salt (32%, P<.01) diets. Fractional sodium and bicarbonate excretion based on inulin clearance were likewise significantly lower in the salt-sensitive individuals after sodium citrate intake during both diets (P<.05), pointing to increased sodium and bicarbonate reabsorption. In contrast, net acid excretion after ammonium chloride was not different between the two groups. Our finding of an enhanced bicarbonate reabsorption in salt-sensitive men could indicate a compensatory renal adaptation to metabolic acid overproduction. This perturbation of renal bicarbonate excretion may contribute to sodium retention and thus salt sensitivity in genetically susceptible humans. (Hypertension. 1993;22:884-890.)

KEY WORDS • hypertension, sodium-sensitive • acid-base equilibrium • sodium • ammonium chloride • ion exchange • metabolism • diet, sodium-restricted

A perturbation of cellular electrolyte homeostasis has long been implicated to play a fundamental role in the pathogenesis of essential hypertension. Until recently, however, most studies on electrolyte metabolism have concentrated on the role of the sodium, calcium, potassium, and chloride ions (see Reference 1 for review). The findings of lower intracellular and extracellular pH and bicarbonate levels compared with salt-resistant controls. This observation is in line with the finding of lower intracellular and extracellular pH levels and increased activity of the Na\(^+\)-H\(^+\) antiporter, an important regulator of intracellular acid-base homeostasis, in several rat models of hypertension (see Reference 7 for review). The findings of lower intracellular pH levels in red blood cells\(^8\) and of an increased activity of the Na\(^+\)-H\(^+\) antiporter in platelets\(^9,10\) leukocytes,\(^11,12\) and red blood cells\(^13-16\) from patients with essential hypertension also point to an abnormality of cellular acid-base homeostasis in hypertension.

The kidney plays a major role in the modulation of systemic acid-base balance. The process of bicarbonate generation and reclamation as well as the H\(^+\) secretion function of the nephron are closely linked to sodium homeostasis. The perturbation of acid-base status associated with salt sensitivity thus may involve an alteration of both renal acid-base and sodium homeostasis. Because the kidney can contribute to the development of metabolic acidosis either by a failure to adequately conserve bicarbonate or by a reduced ability to excrete a metabolic acid load, as found in the various syndromes of renal tubular acidosis, in the present study we examined the renal response to an acute alkali or acid load in salt-sensitive normotensive individuals. Normotensive salt-resistant subjects served as controls. The findings of this study demonstrate that renal bicarbonate excretion after the administration of an oral alkali
load is significantly lower in salt-sensitive than in salt-resistant normotensive men, pointing to a perturbation of renal acid-base regulation in these genetically hypertension-prone individuals.

Methods

The protocol of the study was approved by the ethics committee of our hospital. All participants gave their informed consent. The study was performed in an ambulatory setting. All diets were prepared in the hospital kitchen.

Subjects

Twenty-four healthy male volunteers (22 to 29 years old) were studied after routine physical and laboratory examinations to rule out hypertension, hyperlipidemia, diabetes mellitus, or hepatic or renal disease. Only subjects with a diastolic blood pressure below 85 mm Hg and a systolic pressure below 140 mm Hg were included. Parental histories on hypertension were obtained by direct personal communication with family physicians. Subjects with at least one parent under treatment for hypertension were regarded as having a positive familial history of hypertension. According to this definition, 12 subjects had positive familial histories of hypertension and 12 subjects had negative familial histories.

Dietary Regimen

Subjects were given a standardized diet containing 80 g protein, 250 g carbohydrates, 80 g fat, 20 mmol sodium chloride, 60 mmol potassium, and 20 mmol calcium per day for 14 days. Caloric intake ranged between 2000 and 2400 kcal/d according to body weight and physical activity. The subjects were advised to drink approximately 2 L water per day. In a randomized single-blind crossover fashion, a daily supplement of 22 tablets of slow sodium (10 mmol NaCl per tablet, gift of CIBA-GEIGY, Horsham, UK) or placebo was administered for 7 days each. This resulted in a daily salt intake of 240 mmol during the high-salt period, which exceeds average sodium intake in Western European societies by roughly 30%, but is still within the normal range.

Procedures

On the fifth and seventh days of each period, an antecubital vein and the contralateral radial artery were cannulated in the fasting subject. After the administration of an intravenous loading dose of insulin (50 mg/kg, Inutest, Laevosan, Linz, Austria) followed by an insulin infusion (27 mg/min) for the assessment of inulin clearance, blood pressure was measured in the supine subject over 1 hour at 1-minute intervals with an automatic oscillometric device (Dinamap 1846 SX, Critikon, Tampa, Fla). After blood pressure was measured, subjects emptied their bladders by spontaneous voiding. A crossover fashion, a daily supplement of 22 tablets of slow sodium, which was given tap water corresponding to the amount of urine passed throughout the urine collection period. In 15 subjects, six of whom had positive familial histories of hypertension, the above protocol was performed only on the seventh day of each dietary period with the administration of sodium bicarbonate only.

Throughout the study, dietary compliance was assessed by measuring daily 24-hour urinary sodium, chloride, potassium, and creatinine excretions. Subjects were considered compliant when sodium and chloride excretions were less than 35 mmol per 24 hours and greater than 200 mmol per 24 hours by the third day of the low- and high-salt periods, respectively.

Laboratory Procedures

Plasma and urinary sodium and potassium concentrations were measured with an ionometer EF (Freseinius, Bad Homburg, FRG), chloride with a Chloride Analyzer 925 (CIBA-Corning, Fernwald, FRG), and inulin with a standard photometric method using anthron reagent. Arterial and urinary pH and PCO₂ were measured with a blood gas system (Radiometer, Copenhagen, Denmark). Bicarbonate was calculated according to the Henderson-Hasselbalch equation, whereby the pK₂ of carbonic acid in urine was calculated as pK₂=6.33−0.5([Na]+[K])⁻¹² (expressed as equivalents) to compensate for variations in ionic strength. Urinary titratable acidity was calculated from the amount of 0.1 mol/L NaOH used to titrate a 1-mL urine sample up to a pH of 7.4. Urinary ammonium was measured by a formalin titrimetric method. Net acid excretion was calculated as the sum of titratable acidity and ammonium minus bicarbonate excretion. Fractional excretion of sodium and bicarbonate was calculated based on inulin clearance. Plasma aldosterone concentration was measured by radioimmunoassay (CIS Bioindustries, Gif-sur-Yvette, France).

Assessment of Salt Sensitivity

As in previous studies, salt sensitivity was defined as a significant drop in mean arterial pressure (>3 mm Hg) during the low-salt diet calculated as the difference between the average of the 60 readings on the seventh day of the high- and low-salt periods (P<.05, two-tailed t test for independent samples). The SEM for a single 60-minute period ranged between 0.40 and 0.95 mm Hg. Subjects whose blood pressure was not significantly affected by salt intake were considered salt resistant. We have previously shown salt sensitivity defined thus to be a well-reproducible phenomenon in normotensive individuals.

Data Analysis

Statistical analysis was performed using the spss/pic+ software package (SPSS Inc, Chicago, Ill). Data are reported as mean±SEM. Differences were considered significant at a value of P<.05. Two-tailed Student's t tests for independent and paired samples were used to analyze the between- and within-group effects of the
dietary regimens on physical, blood, and urine variables. Between- and within-group differences over time after the sodium citrate and ammonium chloride loads were assessed by two-way repeated-measures analysis of variance for time-related changes.

Results

Nine of the 13 subjects considered salt sensitive according to the above definition had a positive familial history of hypertension, whereas only 3 of the 11 salt-resistant subjects had positive familial histories. There was no difference in age (25.3±0.5 versus 25.0±0.7 years) or body mass index (22.9±0.5 versus 23.0±0.5 kg/m²) between the salt-sensitive and salt-resistant group.

Administration of sodium citrate resulted in a rapid rise in blood pH and bicarbonate levels and increased renal bicarbonate excretion in all subjects. Although there were no significant differences in the arterial pH or bicarbonate levels between the two groups after the sodium citrate load (Fig 1), cumulative bicarbonate excretion was markedly lower in the salt-sensitive than in the salt-resistant subjects during both the low-salt (46%, P<.001) and high-salt (32%, P<.01) diets (Fig 2). Fractional bicarbonate excretion after sodium citrate was likewise lower in the salt-sensitive than in the salt-resistant subjects during both diets (P<.05) (Fig 3). Titratable acidity and ammonia excretion after sodium citrate were not different between the two groups. Administration of sodium citrate also resulted in a marked rise in urinary sodium excretion in both groups (Fig 2), whereby fractional sodium excretion was lower in the salt-sensitive subjects than in their salt-resistant counterparts (P<.05) (Fig 3). Administration of ammonium chloride resulted in a similar significant fall in blood pH and bicarbonate levels in all subjects (data not shown). This was accompanied by a marked and comparable rise in net acid excretion in both the salt-sensitive (n=7) and salt-resistant (n=8) individuals (Fig 4).

Although there was no significant difference in blood pressure between the salt-sensitive and salt-resistant subjects during the low-salt diet (Table), blood pressure during the high-salt diet was higher by definition in the salt-sensitive than in the salt-resistant individuals. Urinary and blood variables changed as expected during the dietary regimens and were not significantly different between the two groups (Table).

Discussion

The principal finding of this study was that renal bicarbonate excretion after the administration of sodium citrate was markedly lower in salt-sensitive than in salt-resistant subjects despite a comparable rise in plasma bicarbonate levels. This was true during both a low- and high-salt diet. Reduced renal bicarbonate excretion in the salt-sensitive individuals was associated with a significantly lower fractional sodium and bicarbonate excretion, pointing to increased tubular sodium and bicarbonate reabsorption. In contrast, renal acid excretion in response to ammonium chloride was not different between the two groups.

How can the lower renal bicarbonate excretion relate to the previously reported finding of mild metabolic acidosis in salt-sensitive subjects? A primary increase in renal bicarbonate reabsorption would be expected to lead to a rise in blood bicarbonate levels, resulting in metabolic alkalosis as found in syndromes of metabolic alkalosis of renal origin. Extracellular pH and bicarbonate levels in our salt-sensitive subjects, however, did not reveal the presence of metabolic alkalosis. If altered at all, under baseline conditions they tended to be lower in these subjects, and, in a larger group of salt-sensitive subjects, we have in fact demonstrated significantly lower pH and bicarbonate levels than in salt-resistant
control subjects. Thus, it is unlikely that the observed decreased bicarbonate excretion in the salt-sensitive subjects resulted from a primary renal abnormality. Several mechanisms, including potassium depletion, reduced glomerular filtration rate, and volume contraction, are well known to decrease renal bicarbonate excretion. However, in the absence of a significant difference in either plasma potassium levels, inulin clearance, or salt-induced weight gain between the salt-sensitive and salt-resistant subjects, it appears unlikely that these factors can account for our findings. Another factor that is well known to decrease bicarbonate excretion is acidosis of prerenal origin. Both respiratory acidosis and an increased endogenous or

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**Figure 2**. Bar graphs show cumulative urinary bicarbonate and sodium excretion after administration of sodium citrate in 11 salt-resistant (—) and 13 salt-sensitive (——) normotensive subjects on low- or high-salt diet (mean±SEM). ANOVA, analysis of variance.

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**Figure 3**. Line graphs show fractional bicarbonate (FE bicarbonate) and sodium (FE sodium) excretion after administration of sodium citrate in 11 salt-resistant (○) and 13 salt-sensitive (●) normotensive subjects on low- or high-salt diet (mean±SEM). ANOVA, analysis of variance.
exogenous acid load are compensated for in part by increased bicarbonate reabsorption. Thus, in the absence of metabolic alkalosis, our finding of decreased bicarbonate excretion in salt-sensitive individuals would be compatible with the assumption that renal bicarbonate reabsorption is increased in these individuals in response to an increased systemic metabolic acid production.

The assumption that increased bicarbonate reabsorption in salt-sensitive individuals may be due to increased systemic metabolic acid production is supported by the recent observation of markedly increased acid excretion in Dahl salt-sensitive rats, a widely studied genetic model of salt-sensitive hypertension.²⁴ In that study, net acid excretion in the salt-sensitive strain, which did not exhibit systemic acidosis, was substantially higher during

**Physical, Blood, and Urine Variables in Salt-Resistant and Salt-Sensitive Normotensive Subjects During Administration of Low- and High-Salt Diet**

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Low Salt (n=11)</th>
<th>High Salt (n=11)</th>
<th>Low Salt (n=13)</th>
<th>High Salt (n=13)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Physical variables</strong></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Systolic BP, mm Hg</td>
<td>110.0±2.4</td>
<td>112.2±2.4</td>
<td>112.7±2.1</td>
<td>121.6±2.1*</td>
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<tr>
<td>Diastolic BP, mm Hg</td>
<td>63.8±2.5</td>
<td>61.5±2.4</td>
<td>64.6±3.2</td>
<td>70.0±3.2*</td>
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<tr>
<td>Mean BP, mm Hg</td>
<td>81.4±1.6</td>
<td>80.7±1.7</td>
<td>82.3±2.1</td>
<td>88.8±2.1*</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>57.2±5.8</td>
<td>54.4±1.5</td>
<td>62.5±3.3</td>
<td>60.2±2.4</td>
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<tr>
<td>Weight, kg</td>
<td>75.6±2.9</td>
<td>76.7±3.0</td>
<td>76.9±2.3</td>
<td>77.6±2.2</td>
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<tr>
<td><strong>Arterial blood gas analysis</strong></td>
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<td></td>
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<tr>
<td>pH</td>
<td>7.431±0.007</td>
<td>7.427±0.010</td>
<td>7.423±0.007</td>
<td>7.419±0.005</td>
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<td>Bicarbonate, mmol/L</td>
<td>25.3±2.4</td>
<td>24.9±2.6</td>
<td>25.5±2.6</td>
<td>25.0±2.6</td>
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<tr>
<td>Pco₂, mm Hg</td>
<td>39.0±1.3</td>
<td>39.1±0.7</td>
<td>39.4±1.1</td>
<td>39.4±1.0</td>
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<tr>
<td>Po₂, mm Hg</td>
<td>92.4±2.3</td>
<td>94.6±2.1</td>
<td>100.3±2.9</td>
<td>98.8±2.3</td>
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<td><strong>Plasma variables</strong></td>
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<tr>
<td>Sodium, mmol/L</td>
<td>136.0±0.8</td>
<td>136.6±1.0</td>
<td>134.2±0.5</td>
<td>136.5±0.7*</td>
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<tr>
<td>Chloride, mmol/L</td>
<td>100.3±0.8</td>
<td>103.9±0.9*</td>
<td>101.9±0.7</td>
<td>103.5±0.7*</td>
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<tr>
<td>Potassium, mmol/L</td>
<td>4.07±0.07</td>
<td>4.07±0.03</td>
<td>4.00±0.08</td>
<td>4.10±0.07</td>
</tr>
<tr>
<td>Aldosterone, nmol/L</td>
<td>1.09±0.25</td>
<td>0.16±0.1*</td>
<td>0.86±0.14</td>
<td>0.23±0.04*</td>
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<tr>
<td><strong>Urine variables</strong></td>
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<tr>
<td>Volume, mL/24 h</td>
<td>1497±160</td>
<td>1758±248</td>
<td>1460±151</td>
<td>1975±161</td>
</tr>
<tr>
<td>Sodium, mmol/24 h</td>
<td>22.1±6.0</td>
<td>218±19*</td>
<td>21.9±4.6</td>
<td>234±20*</td>
</tr>
<tr>
<td>Chloride, mmol/24 h</td>
<td>23.8±3.4</td>
<td>214.6±18.3*</td>
<td>23.0±4.3</td>
<td>247.3±15.3*</td>
</tr>
<tr>
<td>Potassium, mmol/24 h</td>
<td>54.8±2.5</td>
<td>61.1±4.6</td>
<td>61.7±4.6</td>
<td>76.0±8.2</td>
</tr>
</tbody>
</table>

BP indicates blood pressure; and bpm, beats per minute. Values are mean±SEM.

*P<.005 vs placebo.
a low-, normal-, or high-salt diet than in salt-resistant controls. This was true even under strict pair-feeding conditions controlling for dietary protein intake. The absence of systemic acidosis in this model of salt-sensitive hypertension suggests that the increased metabolic acid production was fully compensated for by increased bicarbonate reabsorption and acid excretion. The hypothesis that the perturbation of acid-base status associated with hypertension is due to an increase in metabolic acid production rather than to a primary renal abnormality is further supported by the finding of intracellular acidosis not only in Dahl salt-sensitive rats but also in thymic lymphocytes from spontaneously hypertensive rats (SHR), and in red blood cells from patients with essential hypertension, although some authors have failed to confirm these findings.

Intracellular acidosis should result in an increased activity of the Na⁺-H⁺ antiporter, and this has in fact recently been described in circulating blood cells from patients with essential hypertension and in lymphocytes and platelets from the SHR. Careful studies of the kinetic properties of the Na⁺-H⁺ antiporter of platelets from patients with essential hypertension and from lymphocytes from SHR have demonstrated that these properties were not different from those of their respective normotensive controls, suggesting that the increased activity of the Na⁺-H⁺ antiporter was most likely the physiological response to reduced intracellular pH rather than a primary abnormality. Reduced extracellular pH and bicarbonate levels have been described in the SHR, the Milan hypertensive rat, and the stroke-prone SHR, and an increased activity of the Na⁺-H⁺ antiporter (the expected response to metabolic acidosis) has been found in the renal brush border membrane of SHR and Milan hypertensive rats and in the S1 segment of the proximal convoluted tubule of young SHR. Taken together, these findings support the assumption that the perturbation of acid-base status found in our salt-sensitive subjects was more likely due to increased metabolic acid production than the result of a defect in renal acid excretion. Despite the attractiveness of this hypothesis, we would like to point out that in our present study, renal acid excretion during the 2-hour baseline period was not different between the two groups. Therefore, further studies to evaluate urinary acid excretion over a longer period will be required to establish whether increased acid production occurs in salt-sensitive subjects.

How can a decreased bicarbonate excretion as found in our salt-sensitive subjects relate to salt sensitivity? Eighty-five percent to 90% of filtered bicarbonate corresponding to approximately 4500 mEq/d is reclaimed by the proximal tubule. This process, although incompletely understood, is believed to be primarily due to the activity of the Na⁺-H⁺ antiporter located in the luminal brush border membrane. In exchange for sodium, protons are secreted into the tubular lumen, whereby bicarbonate is generated in the cell and returned to the blood. Thus, the proximal tubular Na⁺-H⁺ antiporter is also responsible for the reabsorption of a significant amount of sodium. As noted above, increased activity of the Na⁺-H⁺ antiporter has in fact been found in the S1 segment of the proximal renal tubule of young SHR and in renal brush border membrane preparations from SHR and Milan hypertensive rats, two models of hypertension that were also found to have lower extracellular pH and bicarbonate levels than their respective normotensive controls. This line of reasoning suggests the possibility that increased bicarbonate reabsorption resulting from increased activity of the renal Na⁺-H⁺ antiporter could lead to a concomitant rise in sodium reabsorption, resulting in sodium retention. This idea is in line with our finding that sodium excretion after the administration of sodium citrate tended to be lower in the salt-sensitive group by a magnitude similar to that of the reduction in bicarbonate excretion (Fig 2). Reduced fractional bicarbonate excretion in the salt-sensitive individuals was also accompanied by reduced fractional excretion of sodium (Fig 3). Furthermore, several lines of evidence indicate increased proximal tubular sodium absorption in salt-sensitive normotensive individuals and in offspring of hypertensive parents. Thus, we may speculate that increased bicarbonate reabsorption could lead to sodium retention, thereby contributing to a rise in blood pressure in salt-sensitive individuals. Nevertheless, the possibility that the decreased bicarbonate excretion observed in the salt-sensitive subjects is unrelated to the pathogenesis of salt sensitivity must also be considered.

In summary, we found that bicarbonate excretion after the administration of a sodium citrate load was lower in salt-sensitive than in salt-resistant subjects. In the absence of systemic alkalosis, increased renal bicarbonate reabsorption, most likely indicates a renal compensatory adaptation to metabolic acid overproduction. Increased renal sodium bicarbonate reabsorption, resulting from increased activity of the renal Na⁺-H⁺ antiporter, could contribute to renal sodium retention in these salt-sensitive individuals. This line of reasoning is supported by the finding of intracellular and extracellular acidosis, increased activity of the Na⁺-H⁺ antiporter, and increased net acid excretion in some models of hypertension in the rat. Further studies examining the role of altered renal acid-base handling in patients with salt-sensitive hypertension are clearly warranted.

Acknowledgments

This study was supported by a research grant from the Deutsche Forschungsgemeinschaft (Sh 35/1-1). We would like to thank Hannnlores Brünnecke for expert technical assistance in analyzing the urine and plasma samples.

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Hypertension. 1993;22:884-890
doi: 10.1161/01.HYP.22.6.884

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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