Failure of NaHCO₃ and KHCO₃ to inhibit renin in the rat

THEODORE A. KOTCHEN, JOHN H. GALLA, AND ROBERT G. LUKE
Department of Medicine, University of Kentucky College of Medicine,
Lexington, Kentucky 40506

Kotchen, Theodore A., John H. Galla, and Robert G. Luke. Failure of NaHCO₃ and KHCO₃ to inhibit renin in the rat. Am. J. Physiol. 231(4): 1050-1056. 1976. To evaluate the contribution of chloride to NaCl- and KCl-induced renin inhibition, renin responses to NaCl or NaHCO₃ and to KCl or KHCO₃ loading were compared in NaCl-deprived rats. Sodium balance in animals drinking isotonic NaHCO₃ and NaCl for 9 days did not differ (P > 0.40); K⁺ balance was less positive in NaCl-deprived animals (P < 0.005). Plasma renin activity (PRA) in NaCl-loaded rats (16.5 ng/ml per h * 4.4 SE), but not in NaHCO₃-loaded rats (57.2 ± 9.8), was lower (P < 0.005) than in NaCl-deprived controls (44.8 ± 4.7). Renal renin content (RRC) of NaCl but not of NaHCO₃-drinking animals was also decreased (P < 0.02). Both PRA and RRC of KCl- but not of KHCO₃-loaded rats (5 meq K⁺/10 g diet) were lower (P < 0.01) than in NaCl-deprived controls. After acute intravenous expansion with isotonic NaCl or NaHCO₃, increases of plasma volume and plasma K⁺ did not differ (P > 0.05). However, PRA of NaCl-expanded rats (11.8 ± 3.8) was lower (P < 0.05) than in NaHCO₃-expanded animals (29.7 ± 8.5). The failure of NaHCO₃ and KHCO₃ to inhibit renin suggests a role for chloride in mediating the renin responses to Na⁺ and K⁺.

INHIBITION OF RENIN SECRETION by sodium chloride loading is well documented, and this inhibition has been attributed to either a baroreceptor and/or a macula densa mechanism (5). Acute renal arterial infusion of hydrochloric acid (16) or potassium chloride (6, 25) and chronic potassium chloride loading (1, 22) also inhibit renin, and we have recently demonstrated that acute and chronic calcium chloride loading inhibit renin (14). Infusion of potassium chloride, calcium chloride, or hydrochloric acid was associated with a natriuresis, but no change in glomerular filtration rate, renal blood flow, or systemic arterial pressure, suggesting that renin inhibition may be mediated by an intrarenal mechanism related to alterations in sodium transport.

In all these experiments, chloride was the anion consistently delivered with sodium, potassium, calcium, or hydrogen, and on the basis of micropuncture studies a role for chloride transport at the macula densa on the control of renin has been demonstrated (24). However, the potential contribution of the anion to the regulation of renin release from the whole kidney has not been evaluated. The purpose of the present study is to compare the renin responses to both chronic and acute sodium loading with equimolar concentrations of either sodium chloride or sodium bicarbonate in the rat. To evaluate further the potential importance of chloride, in an additional experiment we compared the renin responses to dietary loading with potassium chloride and potassium bicarbonate in sodium chloride-deprived rats.

METHODS

Experiment 1. Eighteen male Sprague-Dawley rats were given free access to deionized drinking water and a low-sodium chloride diet for 1 wk. The diet, obtained from International Chemical and Nuclear Corp., Cleveland, contained 240 μeq potassium, 9 μeq sodium, and 5 μeq chloride per gram (measured by nitric acid extraction). After 1 wk, the animals were divided into three groups of six rats each: group A was maintained on the same low-sodium chloride diet for an additional 9 days; in group B, deionized water was replaced with isotonic sodium bicarbonate; in group C, water was replaced with isotonic sodium chloride. The diets in groups B and C were continued for 9 days, and in both groups all animals drank the allotted 20 ml/day. During this 9-day period animals in groups A, B, and C were maintained in individual metabolic cages, and to assure a similar dietary intake in sodium-loaded animals, rats in group C were fed 1 day behind group B animals according to a paired-feeding schedule. Daily sodium, potassium, and chloride balances were determined for each animal, based on measuring dietary intake and urinary excretion.

Before beginning the balance study, and again after 7 days of sodium loading, tails were clipped to obtain blood for measurement of hematocrit. After 9 days of sodium loading, all 18 animals were sacrificed by decapitation, and blood emanating from the trunk was collected in chilled EDTA containing tubes for the measurement of plasma renin activity (PRA). A single kidney was harvested from each animal for the measurement of renal renin content (RRC). Thigh muscles were obtained for measurement of sodium and potassium content.

Measurements of plasma electrolytes, particularly potassium, are unreliable in blood obtained by decapitation. Consequently, an additional and identically handled 12 animals were pair-fed on a low-sodium chloride...
diet for 1 wk and subsequently were maintained on this diet plus 20 ml/day of either isotonic sodium bicarbonate \((n = 6)\) or isotonic sodium chloride \((n = 6)\) in place of deionized water for 9 days. These animals were then anesthetized with sodium pentathol and sacrificed by exsanguination from aortic puncture. The following measurements were obtained in aortic blood: \(\text{pH}, \text{Pco}_2\), and plasma sodium, potassium, chloride, and creatinine concentrations.

**Experiment 2.** Twenty-four male Sprague-Dawley rats were placed on a low-sodium \((7.0 \mu\text{eq/g})\), low chloride \(< 1.0 \mu\text{eq/g})\), and normal potassium \((391 \mu\text{eq/g})\) diet for 1 wk. A control group with a diet containing no potassium was not feasible; on such a diet food intake was decreased and animals lost weight. All animals had free access to deionized water. After 1 wk, the animals were divided into 3 groups of eight rats each: group D was maintained on the same low-sodium chloride, normal potassium diet for an additional 8 days; in group E, potassium bicarbonate \((5.0 \text{ meq/10 g of diet})\) was added to the synthetic diet for 8 days; an equivalent amount of potassium, administered as potassium chloride \((5.0 \text{ meq/10 g of diet})\) was added to the base-line diet of group F animals. As in experiment 1, animals were fed according to a paired feeding protocol, and daily balances of sodium, potassium, and chloride were obtained. At the conclusion of the balance study, animals were sacrificed by decapitation and blood was collected for measurement of PRA and blood urea nitrogen (BUN) concentration. A single kidney was harvested from each animal for measurement of renal renin content, and thigh muscles were dissected to measure sodium and potassium.

An additional 12 animals were placed on the same low-sodium chloride diet for 1 wk, and then for a subsequent 8 days were maintained on this diet plus either potassium chloride \((n = 6)\) or potassium bicarbonate \((n = 6)\) as described above. The animals were then exsanguinated by aortic puncture for measurement of arterial \(\text{pH}, \text{Pco}_2\), and plasma sodium, potassium, and chloride concentrations.

**Experiment 3.** As part of a micropuncture study comparing chloride transport after acute volume expansion with sodium chloride or sodium bicarbonate (to be reported elsewhere), the effects of acute volume expansion on PRA were also measured. Male Sprague-Dawley rats were allowed free access to a standard rat-pellet diet and tap water until the time of the experiment. Following Inactin anesthesia and preparative surgery and control measurements, animals received an intravenous infusion of either isotonic sodium chloride \((n = 7)\) or isotonic sodium bicarbonate \((n = 8)\), in a volume equal to 10\% of body weight, over 1 h. During the following 45 min, repeat studies of chloride transport were performed, and aortic blood was then obtained for measurement of PRA. To estimate alterations in plasma volume during acute volume expansion, mean hematocrit was determined on each of six femoral arterial blood samples both before and after expansion.

In all experiments, plasma and urine sodium and potassium concentrations were measured with an Instrumentation Laboratory (IL) flame photometer. Muscle sodium and potassium was extracted with nitric acid (15). Plasma creatinine concentration was measured by the method of Kennedy et al. (13), and BUN was measured by the method of Crocker (4). Serum chloride was measured with a Buchler chloridometer (Searle Analytic Inc., Buchler Instruments Division, Ft. Lec, N.J.). An IL blood gas analyzer was used to measure \(\text{pH}\) and \(\text{Pco}_2\); plasma bicarbonate was calculated with a nomogram.

Plasma renin activity was measured in quadruplicate with the radioimmunoassay procedure of Haber et al. (11). For the measurement of RRC, renin was extracted from the entire kidney by the ammonium sulfate precipitation method of Haas (7). An aliquot of this extract was incubated with excess sheep renin substrate, and the concentration of angiotensin I generated after a 15-min incubation at 37°C and \(\text{pH}\) 7.4 was measured by radioimmunoassay. One unit of renal renin is arbitrarily defined as that concentration of renin that will generate 100 ng angiotensin I during the 15-min incubation.

In instances where data were available for only two groups of animals, statistical significance was determined with the Student \(t\) test. When data were compared among three groups of animals, and when the variances for the three groups were similar, the significance of group comparisons was computed with analysis of variance. Because analysis of variance requires similar group variances, for several three-group comparisons with dissimilar variances statistical significance was computed with the Wilcoxon Signed Rank Test (23).

### RESULTS

**Experiment 1.** Overall starting body weight \((\text{mean} \pm \text{SE})\) was 265 ± 5 g. During the experiment, the average weight gain did not differ \((P > 0.10)\) among groups A \((5 \pm 4 \text{ g}), B \((11 \pm 4 \text{ g}), and C \((14 \pm 4 \text{ g})\). After 6 days of a low-sodium diet, and before beginning sodium chloride or sodium bicarbonate loading, the hematocrit of groups B and C \((\text{mean} \pm \text{SE})\) was 53 ± 0.6\%. After 7 days of sodium bicarbonate or sodium chloride loading, the mean hematocrit decreased significantly \((P < 0.05)\) in both group B and group C animals to 47 ± 1.9 and 45 ± 1.0\%, respectively, and these values did not differ \((P > 0.30)\).

In sodium bicarbonate-drinking animals \((\text{group} B)\), at the time of sacrifice, arterial \(\text{pH}\) (Table 1), \(\text{Pco}_2\), and calculated bicarbonate concentration did not differ \((P > 0.40)\) from the respective values in sodium chloride-drinking rats \((\text{group} C)\). Mean plasma creatinine concentration in group B and group C animals did not differ \((P > 0.05)\).

### TABLE 1. Acid-base status and plasma creatinine of NaHCO\(_3\)- and NaCl-loaded rats

<table>
<thead>
<tr>
<th></th>
<th>(\text{pH})</th>
<th>(\text{Pco}_2), mmHg</th>
<th>(\text{HCO}_3) meq/liter</th>
<th>Plasma Creatinine, mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group B, NaHCO(_3) loaded</strong></td>
<td>7.38 ± 0.02</td>
<td>39.9 ± 2.3</td>
<td>24 ± 0.6</td>
<td>1.2 ± 0.1</td>
</tr>
<tr>
<td><strong>Group C, NaCl loaded</strong></td>
<td>7.38 ± 0.02</td>
<td>39.0 ± 1.6</td>
<td>23 ± 0.8</td>
<td>1.0 ± 0.1</td>
</tr>
</tbody>
</table>

Values are means ± SE.
Plasma sodium concentration in group B animals was slightly but significantly ($P < 0.01$) lower than that in group C (Table 2). Plasma potassium and chloride concentrations were also lower in group B animals ($P < 0.001$). Skeletal muscle sodium content did not differ ($P > 0.20$); however, muscle potassium of group B was less than that of group C ($P < 0.01$). Over the 9-day period of sodium bicarbonate or sodium chloride loading, total positive sodium balance in group B and group C animals did not differ ($P > 0.40$). However, over the 9-day period, sodium balance (mean ± SE) in group A animals maintained on the low-sodium chloride diet was slightly negative ($-68 ± 62$ μeq/9 days) and significantly less than that of group B and group C animals ($P < 0.001$). On the final balance day, 24-h urine chloride excretion (mean ± SE) in group A animals compared to that of group C sodium chloride drinking rats ($2,532 ± 17$ μeq/24 h); respective urinary chloride concentrations (mean ± SE) on that day were $4.3 ± 0.8$, $2.0 ± 0.7$, and $232.8 ± 7.9$ μeq/ml. The overall mean ± SE 9-day chloride balance of group C animals ($5,544 ± 796$ μeq/9 days) was significantly greater ($P < 0.001$) than that of group B ($-32 ± 123$ μeq/9 days) and group A ($-222 ± 100$ μeq/9 days) animals. Potassium balance of group B animals was less positive than that of group C ($P < 0.005$).

At the time of sacrifice, mean ± SE PRA (Fig. 1) of sodium chloride-drinking animals ($16.5 ± 4$ ng/ml per h) was significantly less ($P < 0.005$) than that of animals maintained on a low-sodium chloride diet ($44.8 ± 4.7$ ng/ml per h). However, PRA of sodium bicarbonate-drinking animals ($57.2 ± 9.8$ ng/ml per h) did not differ from that of sodium chloride-deprived rats ($P > 0.3$). PRA of sodium bicarbonate-drinking animals ($57.2 ± 9.8$ ng/ml per h) did not differ from that of sodium chloride-deprived rats ($P > 0.3$). In sodium chloride- and sodium bicarbonate-loaded animals, overall, PRA did not correlate significantly with net sodium balance ($P > 0.1$). Total RRC of sodium chloride-loaded animals ($50.4 ± 2.6$ U/kidney) was significantly less ($P < 0.02$) than that of sodium chloride-deprived rats ($P > 0.3$) and was greater than that of sodium chloride-deprived rats ($P < 0.05$). In sodium chloride- and sodium bicarbonate-loaded animals, overall, RRA did not correlate significantly with net sodium balance ($P > 0.1$). Total RRC of sodium chloride-loaded animals ($50.4 ± 2.6$ U/kidney) was significantly less ($P < 0.02$) than that of sodium chloride-deprived rats ($P > 0.3$) and was greater than that of sodium chloride-deprived rats ($P < 0.05$). In sodium chloride- and sodium bicarbonate-loaded animals, overall, RRA did not correlate significantly with net sodium balance ($P > 0.1$). Total RRC of sodium chloride-loaded animals ($50.4 ± 2.6$ U/kidney) was significantly less ($P < 0.02$) than that of sodium chloride-deprived rats ($P > 0.3$) and was greater than that of sodium chloride-deprived rats ($P < 0.05$). In sodium chloride- and sodium bicarbonate-loaded animals, overall, RRA did not correlate significantly with net sodium balance ($P > 0.1$). Total RRC of sodium chloride-loaded animals ($50.4 ± 2.6$ U/kidney) was significantly less ($P < 0.02$) than that of sodium chloride-deprived rats ($P > 0.3$) and was greater than that of sodium chloride-deprived rats ($P < 0.05$).

Net sodium balance (Table 4) of potassium bicarbonate-loaded animals (group E) was slightly but significantly less positive than that of sodium-deprived controls (group D) and potassium chloride-loaded animals was less positive than that of animals on a high-potassium bicarbonate diet ($P < 0.002$). Neither muscle sodium nor muscle potassium concentrations of groups D, E, and F animals differed significantly from each other ($P > 0.05$), although muscle potassium of both potassium-loaded groups tended to be higher than that of controls. Chloride balance of potassium chloride-loaded rate was greater than that of the other two groups ($P < 0.002$). Overall chloride balance in potassium bicarbonate eating and control animals was negative, and potassium bicarbonate-loaded animals were in greater negative chloride balance than controls ($P < 0.002$). On the final balance day, urine chloride concentration was markedly elevated in potassium chloride-loaded animals compared to the other two groups ($P < 0.001$). Plasma renin activity in potassium chloride-loaded animals (group F) was lower than that in group D ($P < 0.02$) and group E ($P < 0.01$) animals (Fig. 2); PRA in sodium chloride-deprived controls (group D) and potassium bicarbonate-loaded rats (group E) did not differ ($P$.

**TABLE 2. Electrolyte balance in NaHCO$_3$ and NaCl-loaded rats**

<table>
<thead>
<tr>
<th>Sodium</th>
<th>Potassium</th>
<th>Chloride</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group B</td>
<td>NaHCO$_3$</td>
<td>139 ± 3.0</td>
</tr>
<tr>
<td>Group C</td>
<td>NaCl</td>
<td>142 ± 3.8</td>
</tr>
</tbody>
</table>

Values are mean ± SE. FFDS, fat-free dried solids. *$P < 0.01$, †$P < 0.001$.

**Fig. 1.** Mean ± SE PRA and RRC responses to NaHCO$_3$ loading and NaCl loading in NaCl-deprived rats.
TABLE 3. Acid-base status, plasma electrolytes, and BUN in KHCO₃ and KCl-loaded rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Na⁺ Balance, meq/100 g FFDS</th>
<th>Muscle Na⁺, meq/liter</th>
<th>K⁺ Balance, meq/8 days</th>
<th>Muscle K⁺, meq/liter</th>
<th>Cl⁻ Balance, meq/liter</th>
<th>Urine Cl⁻, µeq/ml</th>
<th>RRC, %</th>
<th>Total RRC, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>478 ± 51</td>
<td>7.6 ± 0.2</td>
<td>2.070 ± 1.040</td>
<td>42.22 ± 0.78</td>
<td>116 ± 18</td>
<td>2 ± 0.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group D, KHCO₃ loaded</td>
<td>102 ± 80</td>
<td>7.6 ± 0.3</td>
<td>22.430 ± 1.139</td>
<td>44.94 ± 0.77</td>
<td>715 ± 34</td>
<td>3 ± 0.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group F, KCI loaded</td>
<td>370 ± 103</td>
<td>6.8 ± 0.2</td>
<td>32.279 ± 2.816</td>
<td>44.05 ± 1.01</td>
<td>16.493 ± 1.410</td>
<td>362 ± 18</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE. FFDS, fat-free dried solids. *Final balance day.

> 0.1). Overall, in group E and F animals, PRA did not correlate significantly with the 8-day sodium balance (P > 0.1). Total RRC of group F animals was lower than that of group E (P < 0.05), and RRC of group D and group E animals did not differ (P > 0.4). These relative differences were also maintained after adjusting total RRC for differences in kidney weight.

Experiment 3. Conceivably, failure of sodium bicarbonate and potassium bicarbonate to inhibit renin might have been related to subtle differences of plasma volume compared to chronic expansion with chloride. Consequently, PRA was measured after acute volume expansion with sodium chloride or sodium bicarbonate to determine if greater renin inhibition also occurs with sodium chloride expansion, in an experimental situation in which plasma volume was more carefully controlled and was undoubtedly considerably expanded in both groups (Table 5). Bicarbonate-expanded animals were more alkalotic (P < 0.01). Plasma volume expansion, estimated by reduction of arterial hematocrit, did not differ significantly (P > 0.6) in sodium chloride- and sodium bicarbonate-expanded animals. Plasma potassium concentrations of sodium chloride- and sodium bicarbonate-expanded animals also did not differ (P > 0.7). However, PRA of sodium chloride-expanded animals was significantly lower (P < 0.05) than that of animals infused with sodium bicarbonate.

DISCUSSION

Dietary loading with sodium bicarbonate or potassium bicarbonate failed to inhibit PRA and RRC, although the anticipated inhibition of renin was demonstrated after dietary loading with sodium chloride or potassium chloride. The sodium- and potassium-loading experiments were conducted almost 1 yr apart, and PRA of sodium chloride-deprived controls was lower in the potassium experiment. However, appropriate controls were included within each experiment. The difference may, in part, reflect minor differences in the base-line low-sodium chloride diets and the fact that animals in the potassium experiment were older; starting weights (means ± SE) of animals in the sodium- and potassium-loading experiments were 265 ± 5 g and 314 ± 2 g, respectively. In the rat, as well as in man, the renin response to sodium deprivation decreases with advancing age (3, 8). After acute and massive volume expansion with sodium chloride or sodium bicarbonate in the anesthetized rat, PRA was also significantly lower in sodium chloride-expanded animals. Consequently, it is apparent that chloride has an important modulating influence on sodium- and potassium-induced inhibition of renin synthesis and release from the whole kidney.

Changes of hematocrit may not reliably reflect chronic changes in plasma volume, and we cannot exclude minor differences in plasma volume in the chronic experiments. However, it is unlikely that failure of sodium bicarbonate or potassium bicarbonate to inhibit renin can be attributed to a lesser degree of volume expansion compared with sodium chloride- or potassium chloride-loaded animals. Net sodium balance in sodium bicarbonate-loaded animals was considerably more positive than that in sodium chloride-deprived controls, although PRA and RRC did not differ. Comparing animals consuming high-sodium bicarbonate and high-sodium chloride diets, there was no difference of weight gain, decrease in hematocrit, positive sodium balance, or muscle sodium content, suggesting that no major differences of volume expansion occurred between the two groups. Similarly, weight gain, muscle sodium content, and sodium balance of potassium chloride- and potassium bicarbonate-loaded animals did not differ. In

FIG. 2. Mean ± SE PRA and RRC responses to KHCO₃ loading and KCl loading in NaCl-deprived rats.

TABLE 5. Acid-base status, change (Δ) of plasma volume, plasma K⁺, and PRA after acute expansion with NaHCO₃ or NaCl

<table>
<thead>
<tr>
<th></th>
<th>pH</th>
<th>Pco₂, mmHg</th>
<th>HCO₃, meq/liter</th>
<th>Δ Plasma Volume, %</th>
<th>Plasma K⁺, meq/liter</th>
<th>PRA, ng/ml per h</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaHCO₃</td>
<td>7.70 ± 0.02*</td>
<td>25 ± 2†</td>
<td>32 ± 1*</td>
<td>9.2 ± 2.3</td>
<td>3.4 ± 0.2</td>
<td>29.7 ± 8.5†</td>
</tr>
<tr>
<td>NaCl</td>
<td>7.55 ± 0.02</td>
<td>19 ± 2</td>
<td>17 ± 1</td>
<td>10.8 ± 2.5</td>
<td>3.5 ± 0.3</td>
<td>11.8 ± 3.8</td>
</tr>
</tbody>
</table>

Values are means ± SE. Δ Plasma volume was calculated from the following formula: [(Hct_initial - 1)/(Hct_postexpansion)] x 100/(1 - Hct_initial). * P < 0.01. † P < 0.05.
groups of animals loaded with sodium chloride or sodium bicarbonate and potassium chloride or potassium bicarbonate, PRA did not correlate significantly with sodium balance, again suggesting that relatively small differences of sodium balance do not account for the different effects of chloride and bicarbonate on renin. During acute infusion of sodium chloride or sodium bicarbonate, despite considerable and comparable volume expansion and similar mean arterial pressures and glomerular filtration rates, PRA was lower in the group of animals expanded with sodium chloride. Furthermore, because these measurements were similar in both groups of animals, it is also unlikely that the different renin responses to sodium chloride and sodium bicarbonate expansion can be attributed to a baroreceptor mechanism.

Potassium balance in sodium bicarbonate-drinking animals was significantly less positive and both plasma and muscle potassium concentrations were lower, than in pair-fed, sodium chloride-drinking animals. Although dietary potassium depletion in the rat stimulates renin, unrelated to changes in sodium balance (22), it is unlikely that failure of sodium bicarbonate loading to inhibit renin was mediated by an effect on potassium balance. In animals of this age and weight, potassium balance is usually distinctly positive, even when fecal potassium is included in the balance (18) and cumulative potassium balance of potassium bicarbonate-loaded animals was positive (+11.9 meq). Furthermore, the effect of potassium on renin is more prominent in animals consuming a low-sodium diet, and Sealey et al. (22) reported that on a high-sodium intake, dietary manipulation of potassium has little if any effect on plasma renin. In addition, potassium bicarbonate also failed to inhibit the renin response to sodium chloride deprivation, despite a considerably more positive potassium balance than that in either sodium chloride-deprived controls, or the potassium chloride-loaded, renin-suppressed rats of Sealey et al. (22). In the present experiment, sodium balance of potassium chloride-loaded animals tended to be more positive than that of potassium bicarbonate-loaded animals, although this difference was not statistically significant. However, mean sodium balance of potassium chloride-loaded rats was slightly less positive than that of sodium chloride-deprived controls, and renin was suppressed in potassium chloride-drinking rats, indicating that the effect of potassium on renin is not related to sodium balance. Taken together, these results suggest that although potassium balance of bicarbonate-loaded animals was less positive than that of chloride-loaded animals, the failure of sodium bicarbonate and potassium bicarbonate to inhibit renin is not related to relatively small differences of potassium or sodium balance.

Hypokalemia may stimulate renin (12), and plasma potassium concentrations of both groups of bicarbonate-loaded animals were lower than the respective concentrations in chloride-loaded animals. In our laboratory, mean ± SE plasma potassium concentration of rats on a normal potassium, low-sodium chloride diet and maintained under identical balance conditions, is 3.7 ± 0.1 meq/liter, n = 18, a value intermediate between that of both groups of bicarbonate- and chloride-loaded animals. Lower plasma potassium concentrations of bicarbonate-loaded animals presumably reflect an intracellular shift of potassium associated with a higher arterial pH, and indeed, muscle potassium of potassium bicarbonate-loaded animals was slightly higher than that of controls. Consequently, in the chronic loading experiments we cannot exclude the possibility that failure of sodium bicarbonate and potassium bicarbonate to inhibit renin might be related to hypokalemia. However, after acute expansion with sodium chloride or sodium bicarbonate mean plasma potassium concentration did not differ, although PRA was lower in sodium chloride-expanded rats. It is also unlikely that different PRA responses to chloride or bicarbonate loading can be attributed to differences of arterial pH. Although bicarbonate-expanded animals in experiments 2 and 3 were more alkalotic than respective chloride-expanded animals, in experiment 1 arterial pH of sodium chloride- and sodium bicarbonate-loaded animals did not differ, although PRA was suppressed in sodium chloride-loaded rats.

Another possible mechanism for the failure of sodium bicarbonate and potassium bicarbonate to inhibit renin might be related to the effect of the anion on sodium transport at the macula densa. There is increasing evidence to suggest that renin secretion may be inhibited by sodium transport across the macula densa rather than by the concentration of sodium delivered to the macula densa. Vander and Carlson (26) demonstrated that intravenous infusion of furosemide stimulates renin secretion without changing plasma sodium concentration, renal plasma flow, or glomerular filtration rate. Renin stimulation was also not related to volume depletion, and it was suggested that increased renin release was due to inhibition of sodium transport at the macula densa by furosemide. Intravenous injection of furosemide in the rabbit stimulates renin, even if urinary losses are prevented by shunting urine into the femoral vein (17). Similar to furosemide, another loop diuretic, ethacrynic acid, but not chlorothiazide, also stimulates renin release independent of changes in sodium balance. Following release of ureteral occlusion in the dog, there is a natriuresis and renin secretion decreases. However, in animals treated with ethacrynic acid, despite the natriuresis, renin release is either inhibited to a lesser extent or actually increased (2, 9). These experiments provide indirect support for the hypothesis that there is a reciprocal relationship between renin secretion and sodium transport at the macula densa.

In apparent contrast to these whole-animal experiments, which suggest that sodium chloride transport across the macula densa inhibits renin secretion, results of microperfusion studies demonstrate that single-nephron renin activity is increased and single-nephron glomerular filtration rate is decreased in response to sodium chloride transport across the macula densa (24, 28). However, the measurement of single-nephron renin activity may not reflect whole kidney renin release. Indeed, if renin secretion were inhibited by sodium chloride transport across the macula densa, it is con-
ceivable that single-nephron renin activity might be increased acutely due to inhibition of release. An alternative explanation to reconcile results of microperfusion experiments with the present study may be that the effect of sodium transport on renin secretion is modified by the state of sodium chloride load of the animal; single-juxtaglomerular-apparatus renin is not stimulated by microperfusion of the macula densa with saline in saline-drinking rats (24). Furthermore, although the diminution of single-nephron glomerular filtration rate in response to sodium chloride transport across the macula densa is consistent with increased renin release and hence angiotensin production, it is also possible that this feedback mechanism is not regulated by the renin-angiotensin system (19). Despite continuing controversy about the direction of change in renin secretion caused by increased sodium transport across the macula densa, there is agreement that the signal at the macula densa consists of increased transport of sodium with chloride rather than sodium load (9, 28).

Sodium reabsorption at different sites in the nephron may be modified by the anionic composition of the glomerular filtrate (10), and it is possible that the anion accompanying sodium may alter the renin response by affecting sodium transport at the macula densa. In the present chronic loading studies, urinary chloride excretion was approximately 100 times greater in sodium chloride- and potassium chloride-loaded rats than in bicarbonate-loaded animals, and chloride balance was also significantly more positive in these groups than in either controls or bicarbonate-loaded rats. Active chloride transport has been demonstrated in the thick ascending limb of the loop of Henle (1, 20), and the epithelium of the macula densa is morphologically similar to that of thick ascending limb of the loop of Henle (29). Bicarbonate behaves as an unreabsorbable anion in the loop of Henle in the rat (27). We postulate the delivery to the macula densa of sodium with a relatively increased amount of bicarbonate rather than chloride may account for the failure of sodium bicarbonate and potassium bicarbonate to suppress renin.

Results of micropuncture studies during the present acute volume-expansion experiments provide direct evidence for greater delivery of chloride to the distal nephron with sodium chloride expansion than with sodium bicarbonate expansion (unpublished observation). In summary, comparing sodium chloride- with sodium bicarbonate-expanded animals: there were no differences in systemic arterial blood pressure, whole-kidney glomerular filtration rate, single-nephron glomerular filtration rate, filtered sodium load, fractional proximal tubule fluid and, therefore, sodium reabsorption, and urinary sodium excretion. Despite this virtually identical delivery of sodium and water out of the proximal tubule, in animals expanded with sodium chloride, delivery of chloride out of the proximal tubule (mean ± SE, 4.71 ± 0.30 neq/min) was greater ($P < 0.01$) than that in a group receiving sodium bicarbonate (3.44 ± 0.18 neq/min). Schnerrnann (21) has shown that single-nephron glomerular filtration rate feedback is dependent on sodium chloride delivery from the proximal tubule, suggesting that transfer of sodium chloride across the macula densa is responsive to changes in luminal sodium chloride concentration. Urinary chloride excretion in the present experiment was also greater ($P < 0.001$) in sodium chloride-expanded rats (mean ± SE, 7.91 ± 1.53 μeq/min) than in sodium bicarbonate-expanded rats (0.50 ± 0.13 μeq/min). These results are also consistent with the hypothesis that the failure of sodium bicarbonate to inhibit renin may be related to decreased delivery and consequently decreased transport of chloride across the macula densa.

In conclusion, sodium bicarbonate and potassium bicarbonate, in contrast to sodium chloride and potassium chloride, failed to inhibit renin in the rat. Although we cannot exclude a minor effect of volume or differences in plasma potassium concentrations, our results are consistent with the hypothesis that increased sodium chloride transport at the macula densa is associated with inhibition of renin synthesis and release in the sodium- or potassium-loaded rat. The signal produced at the macula densa may be substantially modified by the anion delivered with sodium or potassium.

We gratefully acknowledge the secretarial assistance of Ms. Sharon E. Evans.

This study was supported in part by Grants HL15528 and AM13859 from the National Institutes of Health.

Received for publication 24 November 1975.

REFERENCES


