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Independent Effect of Bicarbonate on Renal Citrate Metabolism[1] (40061)

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Renal citrate metabolism in rat, dog and man appears to be a pH dependent reaction (1–3). In addition to the role played by pH there are data indicating that renal citrate metabolism is affected by bicarbonate ions separate from any effect on blood pH. Simpson (4), for example, has demonstrated that in alkalotic dogs increasing the bicarbonate concentration isohydrically raises the renal citrate clearance. Potassium ions also appear to be important in controlling renal citrate metabolism since hypokalemia decreases renal citrate clearance (5). By contrast, acute administration of KCl although increasing muscle citrate does not appear to alter renal citrate content or excretion (6). Thus, both bicarbonate and potassium seem to regulate citrate metabolism but the interrelationships between them are unknown. To study this problem the blood bicarbonate concentration was altered isohydrically in normokalemic, chronically hypokalemic and acutely hyperkalemic rats and renal citrate content and urinary citrate excretion measured. The results show that bicarbonate, separate from its effect on blood pH, alters renal citrate metabolism at all levels of serum potassium.

Methods. Intact male Sprague–Dawley rats weighing 350–500 g were anesthetized with Inactin and prepared surgically as previously described (7). Unless otherwise noted animals were infused intravenously with 2.1 ml saline per hour and ventilated with a gas mixture containing 30% oxygen and 70% nitrogen. In all experiments a 60 min control period was followed by a 150 min experimental period. Rats were sacrificed by exsanguination from the abdominal aorta into a heparinized syringe. Renal cortical tissue was removed and rapidly frozen in liquid nitrogen for citrate analysis as previously described (5). Each period's urine was filtered and analyzed for creatinine and citrate.

Normokalemic experiments. Following the control period, rats were divided into three bicarbonate groups. The unchanged bicarbonate group, was ventilated with a 30% oxygen–70% nitrogen gas mixture at a rate of 120/min and a tidal volume of 2.0 ml while receiving 2.1 ml of saline per hr. The lowered bicarbonate group was hyperventilated with the 30% oxygen–70% nitrogen gas mixture at a ventilatory rate of 140/min and a tidal volume of 2.25 ml. Simultaneously, the venous infusate was changed to 0.2 *N* HCl in saline given at approximately 2.1 ml/hr. The elevated bicarbonate group, was ventilated with a 10% carbon dioxide, 30% oxygen, 60% nitrogen gas mixture at a rate of 120/min and a tidal volume of 2.0 ml. Simultaneously, the venous infusate was changed to a 0.35 *M* sodium bicarbonate solution given at approximately 2.1 ml/hr. Mean total sodium administered to the normal, lowered and elevated bicarbonate groups in the experimental period was 1.28, 1.28 and 1.46 mEq respectively. In all groups 0.2 ml blood samples were taken at 15 min intervals for the first hour of the experimental period to ascertain constancy of blood pH. This was not done in all animals once the procedure had been standardized. This same protocol was employed in hypokalemic and hyperkalemic animals. Other normokalemic rats were prepared in the same manner but acute respiratory acidosis was induced by ventilation with 10% carbon dioxide, 30% oxygen and 60% nitrogen mixture.

Chronic hypokalemia protocol. Rats were placed on a low potassium, low sodium diet (obtained from Nutritional Biochemical Corporation, Cleveland, OH). Animals were allowed water containing 75 mEq/liter of sodium bicarbonate *ad libitum* during the entire study. Rats were maintained on this diet for 15 days. On each of days 8 through 12 rats were given 2 mg of furosemide intraperitoneally.

Acute hyperkalemia protocol. Rats were tube fed twice on the day prior to the proce-

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dure and once on the morning of the experiment. Each feeding consisted of 2 ml of 1 mg/ml triamterene solution and 10 ml of a 5 mEq/liter potassium chloride solution in 5% dextrose and water. In addition, a solution containing 5 mEq/liter potassium chloride was given intravenously during the experimental period at 6.0 ml/hr.

Analytic methods. Arterial pH and pCO_2 were measured in a Radiometer BMS3 MK-2 blood microsystem and bicarbonate calculated from a nomogram. Renal cortical citrate content, blood citrate, and urinary citrate were measured enzymatically employing citrate lyase (8). Electrolytes and urinary creatinines were determined by flame photometry and standard autoanalyzer techniques.

Results. Blood acid-base and potassium values (Table I). The mean serum potassium concentration of the 24 normokalemic rats shown in Table I was 4.3 ± 0.4 mEq/L in the control period. Control blood pH values did not differ among the three isohydric normokalemic bicarbonate groups and the pH did not significantly change in the experimental period ($P > 0.4$). The blood pH of the hypokalemic and hyperkalemic rats also did not significantly differ among the three isohydric groups ($P > 0.4$) and there were no significant differences between the control and experimental period pH values in any group ($P > 0.5$). The change in bicarbonate concentration in the lowered and elevated bicarbonate groups in each of the three potassium states was highly significant ($P < .001$) at the same time bicarbonate concentration did not significantly alter in the unchanged groups ($P > 0.1$). Serum potassium concentration in the 26 hypokalemic and 19 hyperkalemic rats during the control period was 1.8 ± 0.4 and 7.0 ± 0.4 mEq/L respectively.

Effect of bicarbonate concentration on blood citrate concentration. Isohydric changes in blood bicarbonate did not affect the blood citrate concentration. As shown in Table I although blood citrate rose in the experimental period in all the normokalemic and hypokalemic bicarbonate groups there were no differences in blood citrate among these groups in the experimental period ($P > 0.2$). In hyperkalemic rats blood citrate did not rise in the experimental period ($P > 0.5$) and there were no differences between groups at the end of the experimental period ($P > 0.4$).

Effect of respiratory acidosis on renal citrate content. Acute respiratory acidosis was induced in six normokalemic rats. Blood pH fell from 7.42 ± .02 to 7.06 ± .02 with the acute elevation of pCO_2 from 35 ± 2 to 70 ± 2 mmHg and blood bicarbonate remained unchanged at 22.5 ± 2.2 mEq/L in the control period and 20.6 ± 2.6 mEq/L during the acidotic period. Renal cortical citrate content was 0.132 ± 0.009 μmole/g in the acidotic rats compared to 0.232 ± 0.035 μmoles/g in the normokalemic rats with unchanged bicarbonate.

Effect of bicarbonate concentration on renal citrate content. Renal citrate content in normokalemic rats with unchanged bicarbonate

TABLE I. BLOOD VALUES IN NORMOKALEMIC, HYPOKALEMIC AND HYPERKALEMIC RATS[a].

	Bicarbonate mEq/L		Arterial pH		Citrate μmoles/ml	
Group (n)[b]	Cont.	Exp.	Cont.	Exp.	Cont.	Exp.
Normokalemic						
U (7)	25.0 ± 1.4	24.3 ± 1.6	7.35 ± 0.04	7.34 ± 0.05	0.076 ± 0.020	0.109 ± 0.038
L (10)	21.0 ± 2.4	10.9 ± 2.8[c]	7.36 ± 0.05	7.33 ± 0.05	0.069 ± 0.026	0.116 ± 0.039
E (7)	21.5 ± 1.5	44.3 ± 5.6[c]	7.39 ± 0.02	7.40 ± 0.03	0.081 ± 0.029	0.132 ± 0.060
Hypokalemic						
U (9)	31.1 ± 3.3	27.0 ± 2.9	7.53 ± 0.09	7.52 ± 0.09	0.104 ± 0.045	0.134 ± 0.057
L (11)	37.9 ± 3.5	21.7 ± 4.1[c]	7.51 ± 0.07	7.52 ± 0.06	0.089 ± 0.028	0.144 ± 0.060
E (7)	34.2 ± 1.3	59.8 ± 3.1[c]	7.51 ± 0.04	7.46 ± 0.06	0.091 ± 0.036	0.120 ± 0.082
Hyperkalemic						
U (6)	21.4 ± 2.0	19.9 ± 2.4	7.27 ± 0.04	7.27 ± 0.05	0.057 ± 0.028	0.060 ± 0.022
L (7)	23.5 ± 0.8	13.4 ± 1.1[c]	7.28 ± 0.05	7.27 ± 0.05	0.076 ± 0.034	0.075 ± 0.037
E (6)	20.7 ± 2.7	43.9 ± 5.4[c]	7.29 ± 0.05	7.31 ± 0.03	0.049 ± 0.028	0.069 ± 0.024

[a] All values are expressed as the mean ± SEM. Cont. is control period, exp. the experimental period.

[b] Numbers in parentheses refer to number of animals in each group. U is unchanged, L, lowered, and E, elevated bicarbonate groups.

[c] Significantly different from control ($P < 0.01$).

was 0.232 ± 0.035 μmoles/g compared to values of 0.145 ± 0.016 ($P < .01$) and 0.199 ± 0.022 ($P > 0.2$) in hypokalemic and hyperkalemic unchanged groups respectively. As shown in Fig. 1 at each level of plasma potassium raising the bicarbonate isohydrically increased renal citrate content significantly ($P < 0.001$). Lowering the bicarbonate concentration, however, significantly decreased renal citrate content only in the normokalemic ($P < 0.02$) and acutely hyperkalemic rats ($P < 0.005$).

Effect of bicarbonate concentration on urine citrate excretion. While increased bicarbonate raised urine citrate excretion at each level of potassium (Fig. 2) statistically significant increases were noted only when the lowered and elevated bicarbonate groups at each potassium level were compared ($P < .01$). Figure 2 also shows that as was found with the renal citrate content the greatest absolute changes in urinary citrate excretion occurred in the elevated bicarbonate groups. Respiratory acidosis without change in blood bicarbonate had no effect ($P > 0.4$) on urine citrate excretion with 1.48 ± 0.47 μmoles/mg Crt compared to 1.62 ± 0.6 μmoles/mg Crt in normokalemic unchanged bicarbonate rats.

Discussion. The present experiments clearly show that bicarbonate's effect on renal citrate metabolism, separate from pH, is operative at all plasma potassium levels. These results support the work of Simpson who earlier showed that isohydric increases in bicarbonate concentration raised the urinary citrate clearance of alkalotic dogs (4). However, Simpson was unable to demonstrate a similar effect in acidotic dogs. Our data suggest that his inability to demonstrate any effect of changes in bicarbonate concentrations in acidosis is due to the already markedly decreased urinary citrate clearance in acidosis and thus inability to separate the subgroups. Thus, in our experiments the reduction in urinary citrate excretion induced by lowering bicarbonate in normokalemic rats was not statistically significant while at the same time renal cortical citrate content did decrease significantly in these same animals. Similar results were seen in the hyperkalemic rats where lowering the bicarbonate concentration significantly reduced renal citrate content but not urinary citrate excretion. Only in hypokalemia was a reduction in bicarbonate not associated with a significant decrease in renal citrate content. This may be a consequence of the already significantly reduced cortical citrate content induced by hypokalemia.

The renal citrate content of normokalemic rats with lowered bicarbonate is almost identical to that found in rats with pure respiratory acidosis and a constant bicarbonate concentration. Thus, renal citrate content fell to 0.132 μmoles/g, a value not significantly different from the 0.135 μmoles/g measured in the lowered bicarbonate normokalemic rats or the 0.145 μmoles/g values seen in the unchanged hypokalemic rats. Thus, acidosis without a change in bicarbonate reduces

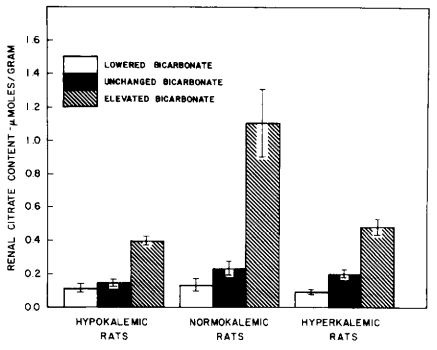

FIG. 1. Renal citrate content in hypokalemic, normokalemic, and hyperkalemic rats. The white bars represent lowered bicarbonate, the black bars unchanged bicarbonate, and the hatched bars elevated bicarbonate groups of rats at each potassium level. Each bar indicates the mean ± SEM.

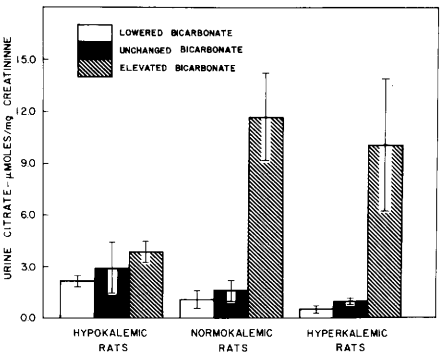

FIG. 2. Urine citrate excretion in hypokalemic, normokalemic, and hyperkalemic rats. The white bars represent lowered bicarbonate, the black bars unchanged bicarbonate, and the hatched bars elevated bicarbonate groups of rats at each potassium level. Each bar indicates the mean ± SEM.

renal cortical citrate content to the same extent as does hypokalemia or an isohydric reduction in bicarbonate concentration.

The experiments also show that urinary citrate excretion increased when the bicarbonate concentration was elevated. This effect, which was demonstrable in each of the three potassium states, could be due to an increased filtered load of citrate, increased renal citrate production, or decreased renal tubular citrate reabsorption and utilization. Since bicarbonate elevation did not alter blood citrate levels, and as urinary citrate excretions were calculated per unit of creatinine excreted thereby correcting for any changes in GFR, the increased urinary citrate cannot be due to an increase in filtered load. It is also unlikely that increased renal citrate production is responsible for the increased urinary citrate. Citrate is primarily produced in intestine and bone (9) and is utilized rather than produced by the kidney. Indeed, in normal acid base and potassium states renal citrate utilization contributes approximately 10% of renal energy needs (10). Also, a variety of experiments have been unable to demonstrate citrate secretion by the mammalian tubule (2). It is probable, therefore, that the increase in urinary citrate is due to decreased renal tubular reabsorption and utilization. Previous clearance studies performed in alkalosis support this conclusion (11). In addition, it has been shown that although incubation of renal cortical slices at varying bicarbonate concentrations does not alter citrate decarboxylation if medium pH is held constant (12), citrate decarboxylation by mitochondria does vary inversely with the bicarbonate concentraiton. From the work of Simpson (13) it has been felt that bicarbonate directly reduces mitochondrial citrate utilization by decreasing the transport of citrate into the mitochondrion. However, the recent work of Robinson *et al.* (14) demonstrated that the decrease in mitochondrial citrate utilization was due rather to bicarbonate increasing citrate efflux from the mitochondria. At high bicarbonate concentration, therefore, less citrate would be metabolized and available for excretion. It seems that the intact animals behave in a fashion similar to mitochondria.

In conclusion, the present experiments add to the growing body of data which show that bicarbonate ions, separate from their effect on pH, alter and regulate metabolic activity (15, 16). This may help explain the greater renal ammoniagenesis found in metabolic acidosis when compared to an identical degree of respiratory acidosis (17). The results thus emphasize the need to control both the pH and the bicarbonate concentration in biological experiments.

Summary. Isohydric alteration of the blood bicarbonate concentration in normokalemic, chronically hypokalemic, and acutely hyperkalemic rats did not affect blood citrate concentration. However, both renal citrate concent and urinary citrate excretion increased in the elevated bicarbonate groups at all potassium levels. Thus, bicarbonate, independent of its effect on pH, affects renal citrate metabolism regardless of the serum potassium level.

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