Effect of chronic NH₄Cl acidosis on proximal tubular H₂O and HCO₃⁻ reabsorption

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LEVINE, DAVID Z., AND LORENA A. NASH. Effect of chronic NH₄Cl acidosis on proximal tubular H₂O and HCO₃⁻ reabsorption. Am. J. Physiol. 225(2) : 380-384. 1973.—Micropuncture experiments were carried out on rats after chronic ingestion of NH₄Cl. At the time of experimentation acidotic animals had a plasma bicarbonate concentration of 14 mEq/liter and displayed signs of extracellular fluid volume depletion (weight loss, increase in plasma protein concentration, and elevated hematocrit). Comparison of proximal tubular function of these rats with control animals showed marked reductions in the absolute net reabsorptive rate for both bicarbonate and water. Acute correction of the metabolic acidosis by administration of NaHCO₃ was associated with a persistence in the defect in water reabsorption and incomplete restoration of bicarbonate retrieval. Neither changes in arterial Pco₂ nor plasma potassium concentration appeared to be significant determinants of the increase in bicarbonate reabsorption associated with correction. We conclude that proximal tubular net fluid efflux does not appear to be rigidly coupled to either the rate of hydrogen ion secretion or the plasma bicarbonate concentration in these experimental maneuvers.

METHODS

Male Sprague-Dawley rats, weighing approximately 250 g, were purchased from Charles River Breeding Laboratories, Inc. and used for all experiments. Control animals were kept on a stock laboratory diet and had free access to food and water until the time of the experiment. Experimental animals were pretreated with NH₄Cl for 2.5 days prior to the experiment. A mixture of 10% ammonium chloride and stock diet was fed ad libitum, and the drinking water contained 140 mEq/liter NH₄Cl. In addition, 18 hr before the experiment these rats were gavaged with approximately 1.6 mEq NH₄Cl per 100 g body wt using a 2 N NH₄Cl solution.

After insertion of the jugular catheters, acidotic animals and control animals received infusions (see below) in the amount of 3% of the body weight per hour. We chose this rate because extensive preliminary experiments indicated that lesser infusion rates were associated with less stable preparations. This was continued throughout the experiment in all protocols employed. In all experiments the infusion proceeded at least 90 min before the first blood or late-proximal micropuncture samples were taken.

Steady-State Experiments and "Acid-Corrected Protocol"

The term acid-corrected protocol refers to micropuncture experiments on four chronic NH₄Cl rats involving a period of metabolic acidosis lasting about 90 min followed by a "corrected" period where plasma bicarbonate was restored to normal by alkali administration. In nine other animals acid base values were determined at 30 min intervals during the period of metabolic acidosis and after correction of the acidosis (hereafter called acid and corrected periods, respectively). In five additional animals of this acid-corrected protocol, plasma potassium concentration was measured. In four animals potassium concentrations were determined at the beginning and end of both the acid and corrected periods; in one animal it was measured at the midpoint of each period.

Correction of the acidosis was effected by intravenous administration of 1 N NaHCO₃ in an amount necessary to restore the plasma bicarbonate concentration from its initial value of about 14 mEq/liter to the normal value of approximately 28 mEq/liter (Fig. 1). Calculation of the bicarbonate deficit was based on a bicarbonate “space” of 50% of the body weight. The 1 N NaHCO₃ was infused over a 2- to 4-min period and an additional period of at least 30 min was allowed to elapse before blood or micropuncture sampling was resumed. During the acid period, the sustaining infusion was 0.9% NaCl, 3% body wt/hr, and during the corrected period an isotonic solution containing bicarbonate (28 mEq/liter) was infused at the same rate.
Acid Sham-Corrected Protocol

To assess the influence of the volume and Na load that were associated with correction in contrast to effects resulting from the change in the acid-base status alone, another protocol (four rats) was employed which was identical to the acid-corrected design except that sham correction was undertaken with 1 N NaCl given as described for NaHCO₃. Also, during the second period of the experiment, 0.9% NaCl was infused rather than the saline-bicarbonate solution. In five of these animals plasma potassium values were determined in the manner described for the acid-corrected protocol.

Data for single or recollection samples of late proximal tubules were derived from 10 control animals receiving an infusion of the saline-bicarbonate solution. Micropuncture samples from 17 rats in metabolic acidosis in many cases were not followed by recollection (see Table 1). These animals were treated exactly as described for the acid period of the acid-corrected and acid sham-corrected protocols. The puncture site of all late-proximal tubules was determined by lissamine green administration. Plasma sodium and potassium concentrations were determined on 10-μl aliquots of plasma using the Radiometer FLM 2 flame photometer. Plasma proteins were determined by a modification of the biuret method. In each experiment 1 mL of inulin-HCl (inulin T, Amershams/Searle) was used, ensuring counts at least 2.5–3 times background despite the use of a portion of the sample for tubular fluid bicarbonate measurements. The scintillation solution used was Aquasol as previously described (4).

All other methodological procedures, including calculations and measurement of tubular fluid bicarbonate concentrations, have been recently detailed (4, 5). Usually not more than six tubules were punctured in each animal and wherever possible t testing was done on paired differences.

RESULTS

After the 3-day period of feeding, drinking, and gavage of the NH₄Cl, body weight decreased in all animals. Thirty-nine animals weighed 258 ± 5.3 g prior to acid induction and 227 ± 4.6 g at the time of the experiment (P < 0.001). Whole-kidney GFR was 0.82 ± 0.05 ml/min per g kidney wt in eight control animals, a value significantly higher than 0.54 ± 0.03 ml/min per g kidney wt in 18 acidotic animals (P < 0.001). In 10 animals in which the acid-corrected protocol was followed whole-kidney GFR increased in eight but this change was not significant, 0.52 ± 0.05 ml/min per g kidney wt (acid) vs. 0.56 ± 0.06 ml/min per g kidney wt (corrected) (P > 0.05). Plasma proteins were 5.7 ± 0.1 g/100 ml in nine control rats and 6.4 ± 0.1 in 10 acidotic animals (P < 0.001). After 90 min of the sustaining infusion, the hematocrit was also found to be significantly higher in the acidotic animals vs. 42.4 ± 1.1% in seven controls (P < 0.001). At the termination of the experiments involving acidotic rats, the hematocrit was still higher than in the control experiments (44.3 ± 0.9% and 38.6 ± 0.9%, P < 0.01), respectively. No apparent difference in whole-kidney GFR was observed as a result of either sham or true correction of the acidosis. Mean GFR in the sham corrected period was 0.56 ± 0.07 ml/min per g kidney wt (P = 5). This value is not different from either the acid phase of the same animals or the true corrected value noted above. Similarly, the hematocrit was 45.5 ± 1.7% (n = 5) after sham correction vs. 43.5 ± 1.3% (n = 7) after true correction, P > 0.05. Blood pressure, measured continuously in all rats, averaged 109.5 ± 1.6 mm Hg in acidotic rats (n = 17) and 110.0 ± 3.2 mm Hg in control rats (n = 10), P > 0.05. Neither sham nor true correction significantly altered these values.

To determine the stability of arterial Pco₂ and plasma bicarbonate concentration, steady-state experiments were carried out in nine rats prepared for micropuncture. Seven of these animals actually provided data for the tubular parameters reported below. Figure 1 demonstrates the constancy of both plasma [HCO₃⁻] and arterial Pco₂ in acid and corrected periods. The Pco₂ values during the corrected period were not different from those of the acid period. Arterial pH was constant in each period (7.23 ± 0.03 at midpoint of the acid period and 7.50 ± 0.02 at midpoint of the corrected period). Also, when comparison was made between acid-base parameters of the two periods of the acid sham-corrected protocol no significant differences were noted. Furthermore, the acid-base values were identical in the acid periods of the true or sham-corrected rats.

In 10 animals plasma potassium concentration was determined during the acid period and during either the corrected period (five rats) or during sham correction (five rats). In every rat, plasma potassium concentrations fell from 0.1 to 0.5 mEq/liter; in the acid-corrected protocol plasma potassium concentration fell from 3.0 ± 0.2 to 2.7 ± 0.2 mEq/liter (P < 0.02), and in the acid sham-corrected rats plasma potassium decreased from 3.4 ± 0.2 to 3.0 ± 0.2 mEq/liter (P < 0.01). Plasma sodium concentration averaged 146 ± 0.9 before and 147 ± 1.8 mEq/liter after true correction (P > 0.05). In the sham-corrected protocol the corresponding values were 146 ± 1.9 and 147 ± 2.2 mEq/liter, respectively (P > 0.05).

Micropuncture Experiments

Control animals. In seven control animals the validity of the recollection technique in our hands was reassessed in 25 tubules. Single-nephron GFR (SNGFR) averaged 83.4 ± 1.7 ml/min during control and 39.9 ± 2.1 ml/min after recollection with no experimental maneuver intervening. Corresponding values for fractional bicarbonate and fractional water reabsorption were 0.68 ± 0.03, 0.67 ± 0.02,
TABLE 1. Summary of micropuncture data

| Comparison of Control and Acidotic Rats (Not Recollection) |
|---------------------------------|-----------------|-----------------|-----------------|
| | Cont | Acid | Cont | Acid | Cont | Acid |
| Mean | 0.33 | 0.33 | 0.68 | 0.65 | 12.7 | 7.9 |
| ±SE | 0.02 | 0.01 | 0.02 | 0.01 | 0.9 | 0.4 |
| N/n | 10/41 | 17/65 | 10/29 | 17/64 | 10/40 | 17/84 |
| Stat | NS | P < 0.001 | P < 0.001 | P < 0.001 |

| Acid-Corrected Protocol (Recollection) |
|---------------------------------|-----------------|-----------------|-----------------|
| | Acid | Corr | Acid | Corr | Acid | Corr |
| Mean | 0.28 | 0.21 | 0.85 | 0.50 | 8.1 | 7.9 |
| ±SE | 0.02 | 0.02 | 0.01 | 0.04 | 0.6 | 0.8 |
| N/n | 4/17 | 4/17 | 4/17 | 4/17 | 4/17 |
| Stat | NS | P < 0.001 | NS | 0.01 | NS | P > 0.002 |

| Acid Sham-Corrected Protocol (Recollection) |
|---------------------------------|-----------------|-----------------|-----------------|
| | Acid | Sham | Acid | Sham | Acid | Sham |
| Mean | 0.33 | 0.34 | 0.87 | 0.80 | 7.2 | 9.6 |
| ±SE | 0.05 | 0.04 | 0.02 | 0.02 | 0.6 | 2.8 |
| N/n | 4/18 | 4/18 | 4/18 | 4/18 | 4/18 |
| Stat | NS | P > 0.02 | NS | NS |

N/n: number of rats/number of tubules. Stat = statistical significance.

and 0.35 ± 0.03, 0.36 ± 0.03, respectively. Recollection values for the absolute net reabsorptive rate for water were 13.9 ± 1.0 and 14.3 ± 1.5 nl/min, while corresponding values for bicarbonate were 811 ± 54 and 834 ± 58 μEq/min. Finally, the recollection values for the ratio of absolute net reabsorptive rates for bicarbonate and water were 60.9 ± 3.5 μEq/nl and 61.8 ± 4.5 μEq/nl, respectively, P > 0.05.

Acidotic animals. Table 1 gives various late proximal values for 17 acidotic rats compared with 10 control animals. Despite a significant fall in SNGFR from 37.1 ± 1.4 nl/min (control, n = 41) to 24.5 ± 0.7 nl/min (acid, n = 45), fractional water reabsorption was identical because of an exactly proportionate fall in absolute water reabsorption. The TF/P HCO₃⁻ ratios were 0.50 ± 0.02 (control, n = 35) and 0.22 ± 0.01 (acidic, n = 82), P < 0.001. The amount of bicarbonate reabsorbed per unit of water removed was 59.9 ± 2.9 μEq/nl (control, n = 29) and 44.8 ± 2.2 μEq/nl (acidotic rats, n = 63), P < 0.001.

Acid-corrected animals. Seventeen late-proximal tubules were sampled during the period of metabolic acidosis and again after acute correction of the acidosis by NaHCO₃ administration. Figure 1 shows the changes in plasma bicarbonate concentration and Pco₂ in these animals. The SNGFR during the acid period was 29.0 ± 1.2 nl/min and increased to 36.6 ± 1.7 nl/min (P < 0.001; n = 17) after correction. Values for the other micropuncture parameters are given in Table 1. Upon correction TF/P HCO₃⁻ increased from 0.23 ± 0.01 to 0.59 ± 0.04, P < 0.001. Absolute bicarbonate reabsorption of corrected animals averaged 596 ± 61 μEq/min, a value which was significantly lower (P < 0.05) than 818 ± 50 μEq/min of control animals, but significantly higher than the value of 401 ± 23 μEq/min, obtained in the initial acid period. The ratio of absolute bicarbonate to water reabsorbed increased from 52.0 ± 4.5 to 80.0 ± 7.6 μEq/nl, P < 0.01. The latter value was significantly different from the control recollection ratio (61.8 ± 4.5 μEq/nl, see above) at the 0.01 level.

Acid sham-corrected animals. Exact replication of the acid-corrected protocol, except for the substitution of chloride for bicarbonate in the solutions used, permitted the separation of possible effects secondary to extracellular fluid (ECF) volume change and Na load from those resulting from alterations in acid-base status. During the initial acid period, SNGFR was 23.0 ± 1.2 nl/min, a value not different from 25.0 ± 3.9 nl/min (n = 18) measured after sham correction. The TF/P HCO₃⁻ ratio increased significantly from 0.19 ± 0.02 to 0.31 ± 0.03, P < 0.001. Other values are given in Table 1. In contrast to the acid-corrected rats, sham correction resulted in no significant differences in fractional water reabsorption, absolute bicarbonate reabsorption, or in the ratio of absolute bicarbonate to water reabsorption. It is also of interest to note that although the differences in the TF/P HCO₃⁻ ratio and fractional bicarbonate were significant, the changes were significantly less (P < 0.001) when compared with the corresponding values of the true corrected periods.

DISCUSSION

The interpretation of our results can best be presented by dividing the discussion into three parts: 1) the dissociation of the rates of absolute net reabsorption of water and bicarbonate, 2) the reduction of absolute net water reabsorption in acidotic rats, and 3) the changes in absolute net reabsorptive rates for bicarbonate.

1) Dissociation of Rates of Absolute Net Bicarbonate and Water Reabsorption

The most interesting finding in our studies is the demonstration that the ratio of absolute amounts of bicarbonate and water reabsorbed by rat proximal tubules can be made to change significantly. When control rats are compared with acidotic rats this ratio is quite different even though fractional water reabsorption is unchanged. If comparison is made between rats whose acid-base status is acutely corrected and either sham-corrected or control animals, this ratio is still significantly different despite the restoration of SNGFR and plasma bicarbonate levels to normal. Thus, in two quite different maneuvers there is apparent separation of the controlling mechanisms for bicarbonate and water reabsorption. This result is consistent with a previous study from this laboratory (4) and of great relevance to recent reports which indicate coupling between sodium re-
absorption and hydrogen ion secretion (3, 12). While our results appear to exclude rigid coupling of all Na transport to bicarbonate efflux and hence hydrogen ion secretion, it is possible that our experimental maneuvers have also inhibited a component of Na transport unrelated to the proposed hydrogen ion secretory mechanism. For example, alteration of glucose or amino acid substrate levels, inhibition of Na- K-activated ATPase by ammonium ion (2, 10) or change in RNA, DNA, or glutaminase levels (1, 6) could conceivably lead to disparate rates of absolute net bicarbonate and water reabsorption in the presence of continued coupling of at least a portion of Na and bicarbonate movement.

2) Reduction in Absolute Net Water Reabsorption

Rats chronically ingesting NH₄Cl show unchanged proximal fractional water reabsorption and markedly reduced absolute net water reabsorption when compared with control animals. Our NH₄Cl-treated animals also displayed unequivocal stigmata of ECF volume contraction but failed to respond in the manner that would be anticipated from the studies of Weencer et al. (13) involving comparably contracted rats. These authors reported significant increases in fractional and absolute water reabsorption when comparison was made with controls. Accordingly, it is likely that some component of chronic NH₄Cl ingestion has led to impaired water reabsorption in our contracted animals. This suggestion has already been made in preliminary reports by Rector and his associates (8, 11) and is consistent with other studies (3, 12) that would predict depressed proximal tubular water uptake if ambient bicarbonate concentrations are low. We have also offered further suggestions (see above) which might readily account for altered water reabsorption. In fact, the results of acute correction of the metabolic acidosis and the parallel sham-correction studies permit us to suggest that the persistence of the defect in water reabsorption is not contingent either upon the low SNGFR or the presence of metabolic acidosis at the time of experimentation. It may be argued that volume expansion associated with correction of the acid-base status prevented restoration of absolute net reabsorption of water to normal levels. The higher hematocrit and low whole-kidney GFR at the end of these experiments (with reference to controls) suggests that this is unlikely. It is also likely that the change in SNGFR noted only in the true-correction protocol reflects some result of the change in acid-base status rather than greater ECF volume expansion. That equimolar and equivolume solutions of NaHCO₃ would appear to be quite reasonable.

3) Changes in Absolute Net Reabsorptive Rates for Bicarbonate

Reasons for the changes in bicarbonate handling in the different protocols may be readily suggested. When the acidotic rats are compared with control animals absolute net reabsorptive rates for bicarbonate are half-normal. This is not unexpected in view of the marked reduction in the load of bicarbonate filtered. Indeed, the fact that acidotic animals demonstrate the ability to reduce proximal tubular bicarbonate concentrations to very low levels indicates that hydrogen ion secretion proceeds against a considerable gradient and, were sufficient bicarbonate substrate filtered, more would be reabsorbed. This view is supported by the protocol in which the acid-base status of acidotic rats is acutely corrected. SNGFR and filtered bicarbonate load return to normal and a marked increase in the absolute net reabsorptive rate for bicarbonate ensues. That this value persists below that seen in control rats again suggests that some factor associated with prior treatment (such as chronic NH₄⁺ ingestion or the chronic acidosis) may be operant. The fact that hematocrit values are higher and GFR is lower than control (see above) might again be taken as evidence that greater ECF volume expansion was not the cause.

Finally, it is pertinent to consider whether changes in plasma potassium concentration or in Pco₂ might account for alterations in bicarbonate handling, since both of these factors have already been implicated in the control of bicarbonate reabsorption (7). Plasma potassium levels were lower than normal in the acidotic rats, probably as a result of the kaliuresis associated with NH₄Cl ingestion (9). Although there was a significant fall in plasma potassium concentration upon correction of the metabolic acidosis, a similar change was observed during sham correction when no increase in absolute net bicarbonate reabsorption resulted. Accordingly, it seems unlikely that plasma potassium changes significantly modulated bicarbonate movement upon correction of the acidosis. Similarly, since arterial Pco₂ did not change as a result of alkali administration, it too cannot be implicated as a controlling factor.

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