Chloride excretion in nephrons of rat kidney during alterations of acid-base equilibrium

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The present study was undertaken to investigate the characteristics of chloride excretion in situations of metabolic alterations of acid-base balance, including infusion of a poorly reabsorbable anion, sulfate, and carbonic anhydrase inhibition. Chloride concentration gradients as well as electrical potential differences were measured along the rat nephron, and fractional reabsorption of this ion was calculated from the simultaneously measured inulin concentration ratios.

METHODS

Male albino rats weighing 250–400 g were used and maintained on a standard laboratory diet containing approximately 1.5% NaCl. One group of rats was maintained on a diet containing 4% NH₄Cl and on 75 mM/liter NH₄Cl as drinking water for 3–5 days prior to the experiments. The rats were anesthetized with 30–40 mg of sodium pentobarbital per kilogram body weight. Control rats received an infusion of isotonic saline at 0.05 ml/min; the group with acutely induced metabolic alkalosis received 5% NaHCO₃ at a rate of 0.1 ml/min beginning 1 hr before the collections; the animals with chronic metabolic acidosis received isotonic NH₄Cl at a rate of 0.05 ml/min; another group received 0.4 mM Na₂SO₄ at 0.1 ml/min, and the last group received a prime of 15 mg/kg acetzolamide (Diamox) and an infusion of 15 mg/kg per hr of this drug in isotonic NaHCO₃ at a rate of 0.1 ml/min. All animals also received a prime and constant infusion of adequate amounts of inulin to maintain a plasma level of 50–100 mg per 100 ml.

The left kidney was exposed by abdominal incision with a lateral extension, and free-flow collections and localizations of the puncture site were made as described previously (12).

Transtubular electrical potential differences were measured on separate groups of rats under similar experimental conditions, by means of glass microelectrodes (12).

Chloride was measured by the microcoulometric method of Ramsay et al. (18) and Windhaber and Giebisch (25). Inulin was determined by a modification of the auhor's method (4, 14). pH in blood and urine was measured by means of a Metrohm model E 399 compensator, with model EA 520 capillary glass electrode. Plasma CO₂ was measured by a manometric Van Slyke apparatus. Blood samples for pH and CO₂ measurement were collected from the abdominal aorta at the end of the experiment.
The influence of Donnan equilibrium and plasma protein concentrations was evaluated experimentally by ultrafiltration through cellophane, a mean ultrafiltrate/plasma ratio for chloride of 1.06 ± 0.009 being obtained in six measurements.

Since frequently a different correction coefficient is used, based on a Donnan factor and correction for plasma proteins, without allowing for chloride binding to this protein, we chose not to correct our values by this factor. Thus, the equilibrium situation for TF/P values is not 1.00, but 1.06 based on a Donnan factor and correction for plasma proteins, without allowing for chloride binding to this protein, we chose not to correct our values by this factor. Thus, the equilibrium situation for TF/P values is not 1.00, but 1.06 based on a Donnan factor and correction for plasma proteins.

RESULTS

A summary of mean values of glomerular filtration rate (GFR), plasma chloride values, chloride/inulin clearance ratios, blood and urine pH, as well as plasma total CO₂ observed in the different experimental groups is presented in Table 1. The respective standard errors are also given.

Data concerning chloride and inulin concentrations along the nephron in control animals, previously published in detail (11, 14), are summarized in Tables 6 and 7, and the range of chloride TF/P as well as U/P ratios with their distribution along the nephron is shown in Fig. 1. Proximal chloride concentrations are considerably higher than the plasma values (mean TF/P = 1.29), also when corrected for Donnan equilibrium and plasma water, when a ratio of 1.06 would be expected. In the distal tubule, on the other hand, TF/P ratios are considerably lower than unity. These data show a distribution similar to that observed by other authors (10, 23, 25). Data for rats undergoing 5% NaHCO₃ infusion are shown in Tables 2 and 6. Chloride TF/P ratios along the nephron are shown in Fig. 1, where it can be noted that in both proximal and distal tubules the distribution is distinctly lower than that of normal rats.

In Table 1, the respective standard errors are also given. Differences between groups could therefore be analyzed by the t test at a significance level of 0.05. This significance level was corrected for repeated comparisons involving the same group of data (e.g., several comparisons with the same control group), using α/N, where α is the chosen level of significance and N the number of comparisons made.

It was shown in this way that both the mean TF/P chloride ratios of proximal tubule (1.08) and distal tubule (0.19) in NaHCO₃-infused rats are significantly lower than control values. Chloride U/P ratios are within the range of control values, despite the presence of an osmotic diuresis, as indicated by an inulin U/P ratio of 1.13 shown in Table 1. This leads to an increased fractional chloride excretion of 2.5% of the filtered load, compared to a value of 0.11% in control animals.

The experimental group with chronic NH₄Cl acidosis (Table 3) showed a mean proximal chloride TF/P ratio of 1.24, very similar to that obtained in control animals. It should be noted, however, that these rats had a high plasma chloride (see Table 1), and the tubular fluid concentrations indicate that bicarbonate must be almost totally absent from this fluid, chloride being practically the only anion at
TABLE 3. Free-flow collections in rats with NH₄Cl acidosis

<table>
<thead>
<tr>
<th>Rat</th>
<th>Loc. %</th>
<th>GFR, ml/kg/min</th>
<th>Inulin TF/P</th>
<th>U/P</th>
<th>TF</th>
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Mean ± SE

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</table>

P = proximal, D = distal.

FIG. 2. Chloride TF/P ratios along nephron in rats with NH₄Cl acidosis.

TABLE 4. Free-flow collections in 0.4 M Na₂SO₄-infused rats

<table>
<thead>
<tr>
<th>Rat</th>
<th>Loc. %</th>
<th>GFR, ml/kg/min</th>
<th>Inulin TF/P</th>
<th>U/P</th>
<th>TF</th>
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Mean ± SE

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<th>U/P</th>
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</table>

P = proximal, D = distal.

this site. Distal chloride ratios are also within the range of control values. Final urine chloride concentrations, on the other hand, are considerably higher than those found in normal rats, leading to a higher fractional excretion rate.

In rats infused with 0.4 M sodium sulfate the reduction of proximal chloride TF/P ratios was not significant, whereas in the distal tubule considerably lower ratios were found, the lowest ratios occurring in final urine (Tables 4 and 6).

The last group, undergoing acetazolamide infusion, show a mean proximal chloride TF/P ratio of 1.09, significantly lower than controls. It is interesting to note that these ratios, as well as those from the bicarbonate-infused group, are...
CHLORIDE EXCRETION AND ACID-BASE EQUILIBRIUM

The distribution of chloride TF/P values along the nephron is shown on Figs. 2, 3, and 4 for NH₄Cl acidosis, sulfate infusion, and Diamox infusion. No definite variation of these ratios along any single nephron segment was detected. In the proximal tubule, values below plasma or the ultrafiltrate/plasma ratio are only seldom found, whereas in the distal tubule these ratios were consistently lower than plasma in all groups studied. Inulin TF/P ratios are summarized in Table 7, where averages of proximal values corresponding to locations between 40 and 65% of tubular length are compared. In the mean corresponding to rats with bicarbonate infusion, five points from three rats not shown in Table 2 are included, since only inulin determinations were made. Only the group with Diamox infusion shows values that are significantly lower than controls (P < 0.05). In the distal tubule, on the other hand, Diamox-infused and acidotic rats show ratios within the % range of controls, whereas animals infused with hypertonic bicarbonate and sulfate present much lower values. These two groups also show the lowest inulin U/P ratios due to the osmotic diuresis induced by their infusion.

Distal transtubular potential differences were measured very similar to the ultrafiltrate/plasma ratio of 1.06 to which reference was made. In this group, also in the distal tubule and in final urine, significantly lowered TF/P ratios of chloride are found (Table 5).

TABLE 5. Free-flow collections in rats with acetazolamide infusion

<table>
<thead>
<tr>
<th>Rat</th>
<th>Loc, %</th>
<th>GER, ml/kg/min</th>
<th>Inulin TF/P</th>
<th>Chloride, mEq/liter</th>
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<td>2.32</td>
<td>1.63</td>
<td>29.3</td>
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<td>P 60</td>
<td>3.08</td>
<td>2.01</td>
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<td>P</td>
<td>3.08</td>
<td>2.42</td>
<td>37.6</td>
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<td>P 16</td>
<td>4.68</td>
<td>1.91</td>
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Mean

P = proximal, D = distal.

TABLE 6. Mean chloride TF/P and U/P ratios

<table>
<thead>
<tr>
<th>Rat</th>
<th>Loc, %</th>
<th>GER, ml/kg/min</th>
<th>Inulin TF/P</th>
<th>Chloride, mEq/liter</th>
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<tr>
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<td>1.63</td>
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<td>P 16</td>
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<td>1.91</td>
<td>19.8</td>
</tr>
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</table>

Mean

P = proximal, D = distal.
concentration ratios along the proximal tubule are not several situations the presence of osmotic diuresis may con-

sorption is offset in all cases by an increased reabsorption in

reduction of this ratio was seen in rats with bicarbonate and

The absence of a variation of these ratios along both proximal and distal tubule indicates that the concentrations measured in the tubular lumen are steady-state concentrations. In the presence of a transtubular potential difference (PD) of about -20 mv, chloride concentrations considerably below plasma level would be expected in electrophysiological equilibrium. If this PD should be an artifact, as suggested by Froemter and Hegel (2), the TF/P ratios observed in the groups with bicarbonate and acetazolamide infusion would be near equilibrium. The same would be the case for the observations of Kashgarian et al. (5) in stationary microperfusions with isotonic raffinose. In our remaining groups, however, the observed concentration ratios, significantly greater than 1.06, are still removed from equilibrium.

Even in conditions of maximal bicarbonate or sulfate availability, chloride concentrations do not fall below plasma levels. On the other hand, as can be seen in Table 3, maximal proximal chloride concentrations are found in rats with chronic metabolic acidosis and NH₄Cl infusion.

The maintenance of a steady-state situation removed from electrochemical equilibrium in the proximal tubule under some of the present experimental conditions could be explained in several ways. In the first place, it is possible that high luminal concentrations are maintained by faster NaHCO₃ than NaCl reabsorption where an equalization of chloride concentrations is prevented by volume reduction of tubular fluid (7). In this case, the flow of water into the interstitium should be faster than the diffusional flow of chloride in the absence of a measurable concentration gradient. In other words, chloride reabsorption at the same rate of water reabsorption would only be possible if assisted by a concentration gradient of approximately 1.2 to 1. In conditions of bicarbonate or Diamox infusion, water flow itself would be slowed or Cl permeability increased suffi-

**Table 7. Mean inulin TF/P and U/P ratios**

<table>
<thead>
<tr>
<th>Group</th>
<th>Prox*</th>
<th>Dist</th>
<th>Urine</th>
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<td>Control</td>
<td>2.41±0.350 (7)</td>
<td>8.64±1.51 (14)</td>
<td>201.2±28.8 (12)</td>
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<td>NaHCO₃, 5%</td>
<td>1.80±0.146 (6)</td>
<td>3.68±0.44 (15)</td>
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<tr>
<td>NH₄Cl</td>
<td>2.06±0.383 (5)</td>
<td>7.35±1.41 (5)</td>
<td>148.2 (3)</td>
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<td>Na₂SO₄, 0.4 M</td>
<td>1.97±0.140 (6)</td>
<td>3.00±0.47 (7)</td>
<td>9.06±0.93 (10)</td>
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<tr>
<td>Diamox</td>
<td>1.69±0.073 (10)</td>
<td>8.46±1.32 (12)</td>
<td>27.4±1.03 (19)</td>
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Numbers in parentheses are number of observations. *Between 40 and 65%.

**Table 8. Distal transtubular potential differences**

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<td>NH₄Cl</td>
<td>-55.0±2.76 (26)</td>
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<tr>
<td>Na₂SO₄, 0.4 M</td>
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<tr>
<td>Diamox</td>
<td>-56.7±1.91 (35)</td>
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</table>

Numbers in parentheses are number of observations.

**Table 9. Fractional chloride reabsorption along nephron**

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<th>Group</th>
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</tr>
<tr>
<td>NaHCO₃, 5%</td>
<td>43.2</td>
</tr>
<tr>
<td>NH₄Cl</td>
<td>43.1</td>
</tr>
<tr>
<td>Na₂SO₄, 0.4 M</td>
<td>42.0</td>
</tr>
<tr>
<td>Diamox</td>
<td>39.1</td>
</tr>
</tbody>
</table>

*Up to 63% proximal length. †Including straight portion of proximal tubule.

**Table 10. Comparison of distal TF/P ratios for Cl calculated by Nernst equation with mean experimental values**

<table>
<thead>
<tr>
<th>Group</th>
<th>Calculated TF/P</th>
<th>Observed TF/P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.17</td>
<td>0.30</td>
</tr>
<tr>
<td>NaHCO₃, 5%</td>
<td>0.14</td>
<td>0.19</td>
</tr>
<tr>
<td>NH₄Cl</td>
<td>0.13</td>
<td>0.34</td>
</tr>
<tr>
<td>Na₂SO₄, 0.4 M</td>
<td>0.16</td>
<td>0.13</td>
</tr>
<tr>
<td>Diamox</td>
<td>0.19</td>
<td>0.16</td>
</tr>
</tbody>
</table>

DISCUSSION

Our data permit an analysis of net chloride reabsorption along the different nephron segments in the investigated experimental situations. The fractional reabsorption of filtered chloride in these segments, based on chloride and inulin concentrations, are shown in Table 9. All experimental groups show a reduced reabsorption of chloride along the proximal tubule with respect to controls. This reduction is maximal in acetazolamide-infused rats. In several situations the presence of osmotic diuresis may contribute to this observation; in the case of acetazolamide a direct action of the drug on NaCl transport at this site may be responsible. This is also indicated by a significantly reduced mean inulin TF/P ratio at the end of the proximal tubule in this group.

It is interesting to note that the reduced proximal reabsorption is offset in all cases by an increased reabsorption in Henle’s loop, including the straight portion of the proximal tubule. This segment or the ascending limb could be responsible for the enhanced reabsorption.

A portion of proximal NaCl reabsorption thus appears to be linked to carbonic anhydrase; it is possible that this reabsorption mechanism is absent in more distal segments, leading to compensation of decreased proximal reabsorption. However, direct action of acetazolamide on NaCl transport independent of its effect on carbonic anhydrase cannot be ruled out.

Distal reabsorption of chloride also appears reduced in several experimental groups, but this may be due to a reduction of the chloride load of this tubular segment.

It had been shown previously (11, 14) that chloride TF/P concentration ratios along the proximal tubule are not changed during the infusion of fursemide, a diuretic that inhibits NaCl transport at this site, nor during 4% NaCl infusion. In the present series of experiments, a definite reduction of this ratio was seen in rats with bicarbonate and acetazolamide infusion, both situations known to increase the bicarbonate concentration of tubular fluid considerably (8).
sufficiently to permit Cl diffusion with a minimal gradient. The establishment of a chloride diffusion gradient by this mechanism could actually represent an additional force leading to Na reabsorption in the proximal tubule, the passive flow of chloride driving that of sodium by a frictional ionic interaction. However, if volume changes should not be adequate to explain the maintenance of these concentrations, as may be the case in zero net volume flow experiments of Khashgarian et al. (6), other mechanisms would have to be invoked. Some evidence for a nonpassive behavior of chloride in proximal tubular epithelium is available (1, 3, 15). A greater unidirectional flow of chloride into the tubular lumen than in the opposite direction may be responsible for the observed concentration levels; it could be due either to enhanced passive flow of Cl due to ionic interaction with the flow of Na or H in the same direction, or to an active transport of chloride. The present data, however, do not give further insight into this problem.

Chloride concentrations in the distal tubule are similar to distal sodium values (13, 25). Mean chloride TF/P ratios were significantly lower than controls in NaHCO₃, NaSO₄- and acetazolamide-infused rats. In Table 10 a lower, but never fall significantly below plasma levels in the equilibrium, most probably due to a reciprocal relationship in tubular epithelium is available (1, 3, 15). Concentrations along the nephron depend on acid-base interaction. However, if volume changes should not be adequate to explain the maintenance of these concentrations, as was observed in metabolic acidosis. Whenever bicarbonate reabsorption is maximal lead to maximal tubular chloride concentrations, as observed in metabolic acidosis. Whenever bicarbonate reabsorption is absolutely or relatively impaired, Cl concentrations are significantly lower, but never fall significantly below plasma levels in the proximal tubule. In the distal tubule, in the latter conditions and during sulfate diuresis, significantly reduced Cl concentrations are found. These data seem to indicate a lower permeability for both bicarbonate and sulfate than for chloride along the nephron, especially in the distal tubule. They could imply that chloride levels along the nephron depend on the relative rate of reabsorption of this anion with respect to the other anions present in the tubular lumen. However, an alternate explanation involving coupling between chloride and Na or H movement into the lumen or an active component of chloride transfer cannot be excluded.

REFERENCES


19. Rector, F., and J. R. Clapp. Evidence for active chloride re-


