Effect of acid lumen pH on potassium transport in renal cortical collecting tubules

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Boudry, Jean F., Larry C. Stoner, and Maurice B. Burg. Effect of acid lumen pH on potassium transport in renal collecting tubules. Am J Physiol 230(1): 239-244. 1976.—In order to determine the effect of acid lumen pH on renal tubular potassium transport, cortical collecting tubules were dissected from rabbit kidneys and perfused in vitro. When the pH of the perfusate was lowered from 7.4 to 6.8, potassium secretion into the tubule lumen decreased by an average of 47%. The transepithelial voltage increased from a mean value of -32 mV (lumen negative) at pH 7.4 to -51 mV at pH 6.8. Net sodium absorption from the tubule lumen was essentially unchanged (5% mean decrease). Transepithelial voltage and potassium secretion returned to control values when the pH of the perfusate was raised to 7.4. Alterations in pH of the bath had no comparable effect on the transepithelial voltage, whether the bath pH was increased or decreased. We conclude that a decrease in the pH of the tubule fluid of itself inhibits active potassium secretion in this tubule segment, providing an additional explanation for the decrease in potassium excretion found in acidosis. The negative voltage (presumably caused by sodium absorption out of the lumen) is increased under these conditions, possibly because of reduction of a smaller counterbalancing positive voltage caused by potassium secretion into the lumen.

urinary acidity; potassium secretion; isolated renal tubules; transepithelial voltage; sodium absorption; amiloride

IN MAMMALIA the potassium filtered at the glomerulus is largely reabsorbed by proximal segments of the nephron (13). Consequently, most of the potassium in the urine is secreted by the distal nephron segments (13, 14, 21). Numerous clearance studies have documented a direct relationship between urinary pH and potassium excretion. For example, metabolic acidosis (15, 18, 21) causes the urine pH and potassium excretion to decrease.1 Conversely, both respiratory (1, 15, 16) and metabolic (15, 21) alkalosis cause the urine pH and potassium excretion to increase, as does administration of the carbonic anhydrase inhibitor acetazolamide (2). Since excretion of potassium in the urine is reciprocally related to urine acidity, Berliner and his collaborators (2, 3) postulated the existence of a distal secretory path-

way in which potassium and hydrogen ions compete for transport in exchange for sodium.

Later, clearance studies by Malnic et al. (15, 16) confirmed the inverse relationship between urinary acidification and potassium excretion in the rat. When they studied the process directly in the distal convoluted tubule by micropuncture, however, they found that hydrogen and potassium ion secretion often varied concordantly in this segment, rather than reciprocally as might have been anticipated from consideration of the urinary changes alone. On this basis, they rejected the previous theory of competition between potassium and hydrogen ions for transport. They hypothesized instead that a decrease in the pH of the epithelial cells caused by the decrease in blood pH or by acetazolamide, was the important factor limiting potassium secretion. They proposed that potassium uptake into the tubule cells from the blood was depressed by the cellular acidosis, causing potassium concentration in the cells to decrease, and that as a result less potassium spilled out of the cells into the urine. This theory differed from that of Berliner in that pH and potassium concentration of the tubule cells was considered to limit potassium excretion rather than competition between potassium and hydrogen ions for transport into the urine.

In the present studies, the technique of perfusion of isolated tubule segments in vitro has been used to determine directly the relationship between acid pH of the tubule fluid and potassium transport in rabbit cortical collecting tubules. The principal result is that when the perfusate pH is lowered, potassium secretion is inhibited. On this basis it is proposed that the decrease in pH of the tubule fluid itself in acidosis may be an important factor limiting potassium secretion.

METHODS

Since the technique used to perfuse isolated renal tubules has been described elsewhere (5-7, 9, 20), only a brief summary is presented here.

Segments of cortical collecting tubules 1.3 to 3.5 mm long were dissected from kidneys of New Zealand white rabbits which had been maintained on standard laboratory chow (NIH feed A). The isolated tubules were transferred to a chamber maintained at 37°C and were perfused with a solution containing, in mM: 150 NaCl; 2.5 K2HPO4; 1.0 CaCl2, and 1.2 MgSO4, titrated to pH 7.4,
6.8, or 5.8 with 0.15 M HCl. Unless otherwise specified, the bathing solution contained 115 NaCl; 5.0 KCl; 25.0 NaHCO$_3$; 10.0 Na acetate; 1.2 Na$_2$HPO$_4$; 1.2 MgSO$_4$; 1.0 CaCl$_2$; 5.5 dextrose; 5% vol/vol calf serum; and was bubbled with 95% O$_2$, 5% CO$_2$ gas to maintain pH 7.4.

Several methods were used to modify the pH of the bath: 1) the bath solution described above was bubbled with either 85% O$_2$,15% CO$_2$, resulting in pH 6.9, or 100% O$_2$ resulting in pH 8.1. 2) The bicarbonate in the bath was replaced in part by chloride so that when the solution was bubbled with 95% O$_2$, plus 5% CO$_2$ the pH was 6.8 for a bicarbonate concentration of 5 mM and 7.0 for a bicarbonate concentration of 10 mM. 3) The pH 7.4 or 6.8 perfusion solution (which contains only phosphate buffer) was used in the bath gassed with 100% O$_2$.

Tubules were perfused using concentric glass pipets as described elsewhere (6). Perfusion rates were 10–15 nl/min, maintained by a perfusion pump (Sage Instruments, Inc., White Plains, N.Y.; model 255-2), or by gravity. The transepithelial voltage was measured between calomel cells connected to the bath and perfusate by agar salt bridges as described previously (6).

Lumen-to-bath fluxes ($J_{lb}$) of sodium and potassium were measured by adding the radioisotopes $^{42}$K and $^{22}$Na to the perfusate. The bath (initially free of radioisotope) was collected for analysis every 10 min. When collecting the bath, 4 ml of fresh solution were washed through the chamber (1.2 ml volume) to insure complete washout of the radioactivity (9). The flux was calculated for each collection using the equation:

$$J_{lb} = \frac{S-b}{S_{b} \cdot L}$$

where $S$ is specific activity of radioisotope in the perfusate, $S_{b}$ is the radioactivity collected in the bath, $t$ is time, and $L$ is the length of the tubule. Since $S_{b}$ was always less than 2% of the radioisotope perfused, the change in the specific activity of the perfusate within the tubule lumen is negligibly small.

Bath to lumen flux ($J_{bl}$) was measured by placing the radioisotopes in the bath and measuring the radioactivity of the collected tubule fluid (initially free of radioisotope): $J_{bl} = (S_{b} \cdot S_{b}^{*})/(S_{b}^{*} \cdot L)$, where $S_{b}^{*}$ is specific activity of radioisotope in the perfusate, $S_{b}^{*}$ is the radioactivity collected in the bath, $t$ is time, and $L$ is the length of the tubule. Since $S_{b}^{*}$ was always less than 2% of the radioisotope perfused, the change in the specific activity of the perfusate within the tubule lumen is negligibly small.

The mean of the results of two to four collections under a given set of conditions was calculated for each tubule. The results for all the tubules were combined and are expressed as means ± standard errors of the means. (The number of tubules is enclosed in brackets.) Statistical significance was estimated using the Student $t$ test comparing paired observations under different conditions in the individual tubules.

RESULTS

Effect of acidic perfusate on ion transport. When the pH of the perfusate was 7.4 (Table 1) there was net secretion of potassium ion into the lumen (bath-to-lumen flux exceeded lumen-to-bath flux) and net absorption of sodium from the lumen (lumen-to-bath flux exceeded bath-to-lumen flux). The absolute values for the unidirectional fluxes are essentially the same as those previously found under similar conditions (20). The flux ratios for both sodium and potassium are high and, as previously noted (20), consistent with active transport of both ion species. When the perfusate was acidic (pH 6.8), the bath-to-lumen flux of potassium decreased significantly without any change in the flux in the opposite direction. The mean decrease in the net potassium flux was 47%. Figure 1 shows the bath-to-lumen potassium fluxes of the individual tubules. As can be seen, the decrease in potassium transport was reversed when the pH of the perfusate was returned to 7.4.

When the perfusate was acidic, there was also a decrease in the lumen-to-bath and net flux of sodium.

<table>
<thead>
<tr>
<th>Table 1. Effect of acidic perfusate on cortical collecting tubules</th>
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<tbody>
<tr>
<td>Control Per fusate (pH 7.4)</td>
</tr>
<tr>
<td>PD, mV</td>
</tr>
<tr>
<td>32.1</td>
</tr>
<tr>
<td>Potassium flux, peq</td>
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<tr>
<td>Bath to lumen</td>
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<tr>
<td>Lumen to bath</td>
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<td>Sodium flux, peq</td>
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$\Delta$, number of tubes. NS, no significant change.
(Table 1 and Fig. 2) without any change in the bath-to-lumen flux. The decrease was relatively small (5%), however, compared to the change in potassium flux (47%) and was equivocal. Since the change is small and equivocal, we are inclined to discount its importance.

The change in potassium flux observed in the present studies could contribute to changes in potassium excretion observed during acidosis. In man, respiratory acidosis, induced by CO₂ inhalation, causes urinary potassium excretion to decrease to one-half its initial value (1). Similarly, in chickens potassium excretion drops to about one-half of its initial value during an infusion of acetic acid into the renal portal circulation (18). In rats studied by micropuncture, metabolic acidosis caused distal tubular potassium secretion to decrease by 80% (15). Since in all the above experiments the pH of the urine (or tubule fluid) decreased during acidosis, it is possible that the change in pH within the tubule lumen contributed to the decrease in potassium excretion, as in the present experiments.

**Effect of acidic perfusate on transepithelial voltage.**

The mean transepithelial voltage under control conditions (pH of the perfusate = 7.4) was -32.1 ± 2.0 mV [12], lumen negative (Table 1), which is similar to the previous result (20) under the same conditions. When the perfusate was acidic (pH = 6.8), the voltage increased significantly (-18.8 ± 1.7 mV [15]) and returned to the control value when the pH was restored to 7.4 (Fig. 3). In other tubules, the pH of the perfusate was lowered to 5.8. The mean control voltage was -29.2 mV. The mean increase in voltage at pH 5.8 was -15.8 ± 1.9 mV [12], virtually identical to the result at pH 6.8. In this respect the results in the rat distal tubule studied by micropuncture are different. The voltage across the distal convoluted tubule of rat did not change during acidosis (15).

The increase in voltage observed in the present studies with the acidic perfusate could have at least two different causes. 1) It could be a diffusion potential resulting from the hydrogen ion gradient across the tissue, or 2) it could be due to the decrease in potassium secretion. As an indirect test of the first possibility, the voltage was measured at different pH's of the perfusate in tubules in which potassium (and sodium) transport was eliminated by poisoning with amiloride. If the increase in voltage with perfusate pH of 6.8 is due to the hydrogen ion gradient, it should still appear under these conditions; but if it is due to changes in potassium transport, it should not. Amiloride, added to the perfusate, caused the voltage to reverse (Fig. 4), as previously noted (20). The positive voltage in the presence of amiloride is believed to be due to hydrogen ion secretion (or bicarbonate reabsorption) which persists when potassium and sodium transport are inhibited. With amiloride present in the perfusate, the voltage was the same whether the pH of the perfusate was 7.4 or 6.8. The lack of change in voltage when the pH of the perfusate was altered in the presence of amiloride is consistent with the conclusion that the hydrogen ion gradient itself does not cause a voltage. The interpretation must be qualified, however, since it is difficult to be certain that the effects of amiloride are limited to sodium and potassium transport. If there were a direct effect of the drug to decrease hydrogen ion permeability, any voltage resulting from the hydrogen ion gradient could be obscured. After removal of amiloride, the voltage became negative again (perfusate pH 7.4) and increased with acidic perfusate (Fig. 4).

**Effect of pH of bath on transepithelial voltage.**

1 For the statistical calculations in Table 1, the control value for each tubule was taken to be either the mean of the values for the initial and final periods at pH 7.4 (when both were collected) or the value for whichever period was collected when the other was not. With this calculation the change in sodium flux at pH 6.8 is statistically significant (P < .05). The change is not statistically significant, however, when the values at pH 6.8 are compared to the values for the preceding periods at pH 7.4 (mean change .37 ± .26 [7] peq cm⁻¹ sec⁻¹) or to the values for the subsequent periods at pH 7.4 (mean change .26 ± .22 [5] peq cm⁻¹ sec⁻¹).
other way to test the effect of hydrogen ion gradient on the voltage is to alter the pH of the bath while keeping that in the lumen constant. To test the effect of an acidic bath, we adjusted the pH of the bath to 6.8-6.9 by three methods: 1) the usual bath containing 25 mM bicarbonate was gassed with 15% CO₂-85% O₂. 2) The bicarbonate concentration was reduced to 5 mM and 5% CO₂-95% O₂ gas was used. 3) The perfusate (phosphate buffer) was used in the bath, at pH 6.8, gassed with 100% O₂. With each method of acidifying the bath, the negative voltage decreased. Pooling the results of all three methods, the mean decrease was +4.6 ± 1.5 mV [15] (< P < 0.01), from a mean control value of -24.3 mV. When the perfusate as well as the bath was changed to pH 6.8, the result was similar to that when the bath alone was changed. With both bath and perfusate at pH 6.8 (bath pH lowered using Methods 2 and 3 above), the voltage decreased by +10.7 ± 2.5 mV [7] (P < .01) compared to the voltage with both solutions at pH 7.4. The decrease in voltage caused by the acidic bath was not reversible. When the pH of the bath (or the bath and perfusate) was restored to 7.4, the mean change in the voltage was -0.1 ± 0.2 mV [9] (or ± 1.0 ± 1.1 mV [7]).

Exposure to the acidic bath apparently damaged the tubules, since after the bath pH had been restored to 7.4 introduction of perfusate at pH 6.8 did not result in a significant change in the lumen negative voltage. The apparent damage to the tubules makes the results with bath pH of 6.8 difficult to interpret. Therefore, a smaller change of the pH of the bath (to 7.0-7.1) was tested in four other tubules. This pH is similar to that of blood in acidotic animals. A pH of 7.0-7.1 in the bath was achieved by replacing 15 mM of the bicarbonate in the normal bath with chloride while still gassing with 5% CO₂-95% O₂. There was no significant change in voltage using this acidic bath. The mean voltages were: precontrol (bath pH = 7.4), -49 mV; acidic bath (pH = 7.0-7.1), -50 mV; postcontrol (bath pH = 7.4), -50 mV.

Thus, in none of the present experiments did an acidic fluid on the peritubular surface of the epithelium have an effect similar to the acidic perfusate which caused the secretion of potassium to decrease.

Douglas and Isaacson (8) previously reported a different result. They found that when the bath was made acidic there was a biphasic change in voltage across rabbit cortical collecting tubules. There was a brief decrease in voltage, followed by a marked increase over a period of 60-180 min. The voltage ultimately became considerably more negative than the control value at bath pH 7.4. Although we routinely observed decreases in voltage during the first 15 min that a collecting tubule was exposed to the acidic bath, no subsequent increase in lumen negativity was seen in tubules exposed to acidic bath for 60-120 min. Thus the results of our experiments, given above, are not in agreement with those of Douglas and Isaacson and we are unable to give the reason. A possible difference was that Douglas and Isaacson maintained the collecting tubules at 25°C, while ours were perfused at 37°C. To test whether temperature might be an important variable, we repeated our experiments in four cortical collecting tubules at 25°C. There was no significant change in voltage when the bath was made acidic (pH 6.8) for 60-120 min at 25°C. Therefore temperature cannot explain the difference between their results and ours.

In other experiments (at 37°C) the effect of an alkaline bath was also tested. When the bath was alkaline (pH 8.1) there was little change in the voltage (+1.8 ± 0.9 mV [4]). The bath was alkalinized by gassing the usual bicarbonate containing buffer with 100% O₂. Perfusate pH in these experiments was 7.4. Neither the results of the amiloride experiments nor the acidification of the bath provides any compelling evidence that the voltage change observed when the perfusate is acified is the result of a diffusion potential. Instead it is likely that the observed voltage change is associated with the observed decrease in potassium secretion.

DISCUSSION

The decrease in potassium secretion found in the present studies when cortical collecting tubules were perfused with an acidic solution provides an additional explanation for the decrease in urinary potassium excretion observed during acidosis. The urine generally is acid during systemic acidosis. Therefore, if low pH of the tubule fluid itself caused potassium secretion to decrease, as found here, this could contribute to the effect. Malnic and co-workers (15) suggested that changes in potassium secretion in the distal convoluted tubules associated with acid-base disturbances are caused by changes in epithelial cell pH rather than tubule fluid pH. Their data show a good correlation between the pH of the tubule fluid and the rate of potassium secretion (Fig. 12 of ref. 15). They chose to discount this, however, since the decrease in potassium secretion in respiratory acidosis was not accompanied by a consistent decrease in tubule fluid pH. Their result during respiratory acidosis certainly suggests that tubule fluid pH is not the sole determinant of potassium secretion in the distal convoluted tubule under these
conditions. It does not exclude the possibility that the tubule fluid pH is an important factor, however, as inferred from the present studies, nor does it provide any direct support for the theory that epithelial cell pH is the most important factor.

The increase in voltage when the perfusate was acidic is most likely due to the change in potassium transport. The negative voltage that normally is present in cortical collecting tubules probably is caused by sodium reabsorption (11, 20). Potassium secretion presumably carries electrical charge in the opposite direction which attenuates or "shunts" the voltage due to sodium transport. Therefore, any reduction in potassium transport increases the opposing voltage and the lumen negativity increases. Inhibition of hydrogen ion secretion with acetazolamide was previously found to have a similar effect on the voltage (20). Other possible causes of the increased voltage seem unlikely. An increase in sodium transport might cause it, but this was not observed. If anything, sodium transport decreased. A reduction in the sodium back flux or an increase in the potassium back flux also could conceivably cause the voltage to increase, but no change was observed in either.

Previously, it was found that there is active potassium secretion in cortical collecting tubules (11, 20). The decrease in potassium secretion that occurred in the present experiments is due to inhibition of the active process. Since the voltage increased, potassium transport should have increased also if it were passive, not decreased as was observed. There was a large change in the potassium flux ratio which is consistent with this interpretation. Potassium flux ratios at the two pH's were calculated from the data in Table 1. Using these flux ratios, the transepithelial voltages ($E_l$) required for the unidirectional potassium fluxes to be independent and passive were also calculated. Table 2 presents these results and compares $E_l$ to the actual measured voltage, $E_m$. With the perfusate at pH 7.4, the ratio of $E_l$ to $E_m$ is 2.3. The ratio is significantly greater than 1, consistent with nonpassive (active) potassium transport, as previously deduced (20). When the pH of the perfusate was 6.8, however, the ratio fell to 1.2, as though no active transport remained.

<table>
<thead>
<tr>
<th>Perfusate pH</th>
<th>Potassium Flux Ratio</th>
<th>Voltage, mV</th>
<th>$E_l/E_m$</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.4</td>
<td>0.062</td>
<td>-74</td>
<td>32.0</td>
</tr>
<tr>
<td>6.8</td>
<td>0.10</td>
<td>-61</td>
<td>51.0</td>
</tr>
</tbody>
</table>

With nonpassive (active) potassium transport, as previously deduced (20). When the pH of the perfusate was 6.8, however, the ratio fell to 1.2, as though no active transport remained.

Distal tubular potassium secretion increases in animals treated with diuretics known to inhibit carbonic anhydrase, such as acetazolamide (2, 17, 21) and certain thiazides (4). Urine pH is also elevated. It is possible that the effect of these drugs to increase potassium excretion is due to the increase in tubule fluid pH, in addition to the previously demonstrated effects of flow rate and sodium delivery to the distal nephron.

In amphibian skin (10, 19) and urinary bladder (12) acidic bathing media cause changes in the transepithelial voltage and sodium transport. The results differ from those in the present studies in which potassium transport was most prominently affected. The difference may reflect different function of the tissues, or different experimental designs. (The anuran membranes were short circuited during the measurements of sodium transport, whereas the collecting tubules were not.)

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Received for publication 19 March 1975

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