Effect of unreabsorbed anions on proximal and distal transtubular potentials in rats

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CLAPP, JAMES R., FLOYD C. RECTOR, JR., AND DONALD W. SELLIN. Effect of unreabsorbed anions on proximal and distal transtubular potentials in rats. Am. J. Physiol. 202(4): 781-786, 1962. - Proximal and distal transtubular potential differences ($E_T$) were measured in Na-fed and Na-depleted rats given infusions of 5% glucose in water, 0.9 M NaCl, 0.5 M NaHCO$_3$, 0.5 M Na$_2$SO$_4$, and 0.25 M Na$_3$Fe(CN)$_6$. Proximal $E_T$ was $-29.8 \pm 0.8$ mV and was uninfluenced by intravenous infusions of various Na+ salts. Distal $E_T$ was $-53.8 \pm 1.0$ mV and rose sharply with infusions of Na+ salts of nonreabsorbable anions; the maximum rise was $-31$ mV in salt-depleted rats given Na$_2$SO$_4$. The rise in distal $E_T$ associated with polyvalent anions was attributed to both higher cation concentrations and lower Cl$^-$ concentrations in distal tubular fluid produced by infusions of these salts. It was suggested that the rise in distal $E_T$ is responsible for the known augmentation of K$^+$ and H$^+$ excretion produced by these salts.

The renal tubule, like other epithelial structures, is characterized by the capacity to develop transcellular potential differences ($E_T$) (1-5). The physiological significance of these has not been completely elucidated. It has been suggested that in the proximal tubule $E_T$ is responsible for the passive reabsorption of Cl$^-$ (6) and the passive secretion of H$^+$ (7).

The significance of the distal $E_T$ in mediating ion transport has not been explored. The role of anions is particularly obscure. The recent demonstration by Rector and Clapp (8) that Cl$^-$ reabsorption in the distal tubule is active suggests that the distal $E_T$ plays little, if any, role in promoting Cl$^-$ reabsorption. However, previous studies have clearly established (9) that the administration to rats of sodium salts of nonreabsorbable anions greatly augments K$^+$ and H$^+$ excretion, strongly suggesting that anions in some manner markedly affect cation transport. It is possible that this effect of nonreabsorbable anions is mediated by changes in distal $E_T$.

The present investigations were undertaken with three broad aims: a) to determine the $E_T$ of the proximal and distal tubule of the rat, b) to ascertain the influence of anions in proximal and distal $E_T$, and c) to determine, if possible, the role of $E_T$ in mediating distal tubular anion and cation transport.

METHODS

Potential differences across the proximal and distal tubule of the rat were measured in normal and salt-depleted animals with or without the acute administration of various sodium salts. Salt depletion was accomplished by giving rats a single dose of chlorothiazide, 15 mg/kg body wt., by gastric tube and then maintaining them on salt-free diets and distilled water for at least 5 days prior to the experiment. Normal rats were given a rat pellet diet and tap water. All animals were allowed free access to food and water up to the time of the experiment.

The rats were anesthetized with pentobarbital, 30 mg/kg body wt. The body temperature was maintained at a normal level throughout the experiment by placing the animal on a thermostatically controlled, electrically heated animal board. Each rat was tracheotomized, and a polyethylene catheter was placed in the external jugular vein just prior to the experiment.

Two types of experiments were performed. In one group of studies, the $E_T$ was measured in either the proximal or distal tubule. A stable potential, lasting as long as 30 minutes, was recorded in some instances; then 1 ml of 0.5 M Na$_2$SO$_4$ was injected intravenously while the potential was continuously recorded. In a second group of experiments, an infusion of various solutions was begun 30 min prior to the initial measurements at a rate of 0.05 ml/min. At the end of 30 min the infusion rate was decreased to 0.02 ml/min and random distal $E_T$'s were measured. The following solutions were infused: a) 5% dextrose in water, b) 0.9 M NaCl, c) 0.5 M NaHCO$_3$, d) 0.5 M Na$_2$SO$_4$, and e) 0.25 M Na$_3$Fe(CN)$_6$.
NaCl, c) 0.5 M NaHCO₃, d) 0.5 M Na₂SO₄, and e) 0.25 M Na₄Fe(CN)₆. In all experiments the infusion resulted in a marked increase in urine flow and dilatation of the distal convolution.

To measure transtubular potential differences the left kidney was exposed using the techniques described by Gottschalk and Mylle (10). Movement of the kidney was reduced by gently lifting it out of the peritoneal cavity and suspending it on a glass ring mounted to the animal board. Special care was taken to avoid traction on the renal pedicle so as to prevent alteration in renal blood flow. Drying of the kidney was prevented by covering it with saline. The kidney was illuminated by a hollow Knisely quartz rod through which saline flowed continuously. To facilitate identification of distal and proximal tubules, 0.5 ml of indigo carmine was injected intravenously 20 min prior to the start of the experiment. (Preliminary experiments revealed that this amount of indigo carmine had no effect on either proximal or distal $E_T$.) Under these conditions, the distal tubules acquire a distinct blue color while the proximal tubules remain colorless.

The measurements of $E_T$ were made with microelectrodes drawn from 1.0-mm Pyrex capillary tubing using a puller similar to that described by Alexander and Nastuk (11). Those electrodes with a long gradual taper were more suitable, because the increased flexibility prevented tearing the tubular epithelium and thereby diminished shunting around the tip of the electrode. The electrodes were filled with 3 M KCl by boiling under reduced pressure for 15-20 min. Only those electrodes with tip resistances between 5-30 megohms and tip potentials less than -10 mV were used. The electrode was inserted into a Lucite chamber filled with 3 M KCl, and electrical contact was established with an Ag–AgCl electrode mounted in this chamber, which connected to the input of a Cary vibrating-reed electrometer. To permit the injection of KCl through the tip of the electrode, the Lucite chamber was equipped with a side arm which connected to a high pressure injection apparatus filled with mineral oil. A reference electrode, consisting of a 3 M KCl bridge in contact with an Ag–AgCl electrode, was inserted into the psoas muscle of the rat.
TRANSTUBULAR POTENTIALS IN THE RAT

783

54.4 mV

0.5 M NaCl
Mean -54.4 mV

NaHCO3
Mean -56.9 mV

Na2SO4
Mean 60.4 mV

Dextrose In Water
Mean -53.8 mV

FIG. 4. Effect of sodium salts of various anions on distal trans-
tubular potential differences in Na+-fed rats.

The measuring electrode was then placed in contact
with the surface of the kidney and the tip potential
balanced out by means of a potentiometer in the reference
side of the electrical circuit. The measuring electrode
was next inserted into the lumen along the longitudinal
axis of the tubule. One minute was allowed for the potential reading to stabilize after the puncture. Only potential readings which remained stable for at least 2–3 min were accepted.

Several methods were used to ascertain that the electrode tip was in the tubular lumen and not in a cell. In all instances the electrode was inserted well along the longitudinal axis of the tubule. Care was taken to place the visible portion of the electrode in the center of the lumen midway between the lateral borders of the nephron. The distal tubular epithelium proved to be very transparent, and the electrode could be seen clearly. This was particularly marked during osmotic diuresis. In some instances it was possible to inject 3 M KCl through the tip of the electrode; when this was done, the KCl could be seen to fill the tubular lumen and the potential would fall abruptly to zero. After the injection, the KCl was washed away by the flow of tubular fluid; the potential then returned to its original level (Fig. 1).

In other instances the electrode was withdrawn slowly through a distance much greater than the diameter of a cell with no significant change in the potential. It was also possible to move the electrode slightly in a horizontal direction and observe the tip to move from one side of the nephron, across the tubular lumen, to the other without changing the potential. The execution of such maneuvers, without significantly altering the potential, indicated that the tip of the electrode was unquestionably in the lumen and not in the cell.

In some instances samples of tubular fluid were collected with micropipettes (tip diameters of 4–8 μ) which were filled with mineral oil stained with Sudan black. The samples were then analyzed for chloride utilizing the method of Ramsay et al. (12), as previously described (8).

After either the potential was measured or the sample of tubular fluid was collected, the site of puncture was identified by injecting the nephron with latex, macerating the kidney for 2 hr in 50% HCl, and then dissecting the nephron free. The localization of the puncture site in the distal or proximal tubule was then ascertained.

RESULTS

Proximal ET

A stable proximal ET was difficult to obtain. In many instances the potential would rapidly rise to as high as -35 mV and then fall to a value of -10 to -15 mV, where it would remain stable for long periods. Such measurements were rejected. Only those readings were accepted where ET remained stable at the highest recorded value.

The values for the proximal tubular ET in normal rats without the infusion of sodium salts are shown in Fig. 2. The mean proximal ET was -23 mV with a standard error of ±0.8. These results are quite similar to those obtained by Solomon in the rat (1) and Giebisch (2) and Whitembury and Windhager (5) in Necturus.

In mice experiments it was possible to obtain a stable

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Na-Fed Rats</th>
<th>Na-Depleted Rats</th>
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|           | No. obs. | Distal tran-
tubular potential diff., mV | No. obs. | Distal tran-
tubular potential diff., mV |
| 5% Dextrose in water | 64 | -59.8±1.9 | 59 | -55.4±1.8 |
| 0.5 M NaCl | 85 | -54.4±1.0 | 61 | -41.6±1.6 |
| 0.5 M Na2HCO3 | 53 | -56.4±1.0 | 40 | -73.0±1.0 |
| 0.5 M Na2SO4 | 100 | -60.4±0.9 | 50 | -73.0±1.0 |
| 0.25 M Na4Fe(CN)6 | 46 | -66.7±1.8 | 63 | -77.0±1.0 |

Values are means ± SE.
proximal ET which remained unchanged after an injection of hypertonic Na$_2$SO$_4$. A typical tracing is shown in Fig. 3.

Measurements of proximal tubular chloride concentrations reveal that, during the infusion of Na$_2$SO$_4$, [Cl$^-$] at the end of the accessible portion of the proximal tubule had fallen only to 40 mEq/liter, so that there was still sufficient chloride present to shunt proximal ET. The failure of Na$_2$SO$_4$ injections to increase proximal ET, therefore, is consistent with the findings of Giebisch that all the chloride in the system must be replaced by SO$_4^{2-}$ to increase the ET (4).

**Distal ET**

In contrast to the proximal tubule, a maximal stable ET in the distal convolution was obtained without difficulty.

**Sodium-fed rats.** The data obtained from normal sodium-fed animals are shown in Fig. 4 and Table I. The mean ET from control rats infused with 5% glucose saline (−53.8 ± SE 1.9), rats given hypertonic NaCl (−54.4 ± SE 1.0, P > .4), and rats given hypertonic NaHCO$_3$ (56.9 ± SE 2.1, P > .4) were not significantly different. However, the infusion of hypertonic Na$_2$SO$_4$ and Na$_4$Fe(CN)$_6$ did result in a significant increase in distal ET over that obtained from control animals. In the group infused with Na$_2$SO$_4$, a mean ET of −60.4 mv ± SE 0.9 (P < .005) was obtained, or an increase over the control mean of −6.6 mv. Rats given Na$_4$Fe(CN)$_6$ had a mean ET of −66.9 mv ± SE 1.8 (P < .005), or an increase over the control ET of −12.9 mv.

In six rats a stable ET was obtained for 10-20 min prior to the injection of Na$_2$SO$_4$. With the electrode still in place the stable potential after the Na$_2$SO$_4$ effect had become manifest was recorded. An illustrative experiment is charted in Fig. 5; Table 2 records the results for all such studies. It is obvious that the Na$_2$SO$_4$ effect is greater when a single distal convolution is continuously monitored than when the ET of rats given Na$_2$SO$_4$ is compared with the ET of control animals. Doubtless this difference results from the fact that internal controls, such as exist in the former type of experiment, more sensitively reflect the influence of Na$_2$SO$_4$.

**Sodium-depleted rats.** The data obtained from the sodium-depleted animals are shown in Fig. 6 and Table I. The distal ET in those rats not given infusions of sodium salts could not be obtained because the distal tubular lumen was usually collapsed, precluding successful puncture. The mean distal ET of the sodium-depleted rats given hypertonic NaCl (−56.6 ± 1.8) was not significantly different from sodium-fed rats given hypertonic NaCl. In contrast to sodium-fed rats, salt-depleted rats responded to hypertonic NaHCO$_3$ with a significant increase in distal ET of −6 mv (P < .005) (Fig. 6). Also, the effects of hypertonic Na$_2$SO$_4$ and Na$_4$Fe(CN)$_6$ were much more striking in the salt-depleted than in the sodium-fed rats (Table 1).

In seven rats stable continuous potentials were recorded from the same nephron before and after the injection of Na$_2$SO$_4$ (Table 3). Na$_2$SO$_4$ resulted in a mean rise in ET of −31 mv, in contrast to the smaller rise of −17 mv obtained in similar experiments on rats maintained on sodium (Table 2).

**DISCUSSION**

The present studies clearly demonstrate that injections of sodium salts of nonreabsorbable anions exert a different effect on ET in the proximal and distal tubule. Intravenous injection of Na$_2$SO$_4$ does not raise proximal ET. This lack of response could readily result from the persistence of enough Cl$^-$ in proximal tubular fluid to exert a constant shunting effect. Giebisch (4) has shown that SO$_4^{2-}$ will increase proximal ET only if all the Cl$^-$ is removed; in our experiments the lowest measured proximal [Cl$^-$] was 40 mEq/liter.

In contrast distal ET rose sharply when Na$_2$SO$_4$ or Na$_4$Fe(CN)$_6$ were administered. In the most refined experiments where a stable ET in a single nephron was continuously recorded, distal ET rose −17 mv after Na$_2$SO$_4$ injection in sodium-fed animals and −31 mv in salt-depleted animals.

The rise in distal ET following injections of sodium salts of nonreabsorbable anions can be attributed to two factors. First, injection of sodium salts of polyanion

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**TABLE 2. Distal transtubular potential differences in same nephron before and after intravenous Na$_2$SO$_4$ injection in Na+-fed rats**

<table>
<thead>
<tr>
<th>Rat No.</th>
<th>Before Na$_2$SO$_4$</th>
<th>After Na$_2$SO$_4$</th>
<th>Change</th>
</tr>
</thead>
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<tr>
<td>Mean</td>
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<td>−17</td>
</tr>
</tbody>
</table>

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**FIG. 5. Effect of intravenous injection of Na$_2$SO$_4$ on distal transtubular potential difference in Na$^+$-fed rats.**
Na₄Fe(CN)₆
Mean -77.4 mV

Na₃SO₄
Mean -73.0 mV

NaHCO₃
Mean -61.0 mV

NaCl
Mean -66.4 mV

FIG. 6. Effect of sodium salts of various anions on distal transtubular potential differences in Na⁺-depleted rats.

Anions results in the presence of more cation per unit volume in the distal tubule than is the case with sodium salts of univalent anions. These differences in cation concentration are due to the different osmotic properties of the various anions. Since tubular fluid reaches isotonicity in the second half of the distal convolution under conditions of maximal antidiuretic activity (as would be the case in these experiments where hypertonic salt solutions were infused), the cation concentration in this area of the nephron would be approximately 150 mEq/liter with Cl⁻, 200 mEq/liter with SO₃⁻, and 240 mEq/liter with Fe(CN)₆⁻. The concentrations in the first half of the distal convolution, before isotonicity has been achieved, would be somewhat lower, but the same ratios would obtain. If, therefore, the distal convolution exhibits any significant permeability to cations, such as appears to obtain in the proximal tubule (4), then the higher cation concentrations associated with polyvalent anions would result in a greater diffusion of positive charges out of the tubular lumen than would occur if univalent anions predominated, thereby raising \( E_T \).

A second factor which probably contributes to the rise in distal \( E_T \) associated with injection of sodium salts of polyvalent anions deserves about the concentration of distal tubular Cl⁻. Previous studies (8) have shown that distal chloride reabsorption is an active process; during Na₂SO₄ diuresis this transport system depresses the concentration of distal tubular Cl⁻ to very low values (less than 1 mEq/liter). Under such circumstances, therefore, the capacity of active Cl⁻-transport to shunt the \( E_T \) would be diminished; consequently higher \( E_T \) would result.

The demonstration that sodium salts of poorly reabsorbed anions elevate distal \( E_T \) affords a reasonable explanation for the effects of these substances on cation excretion. Seldin et al. (9) found that the administration of DOCA plus sodium phosphate or sulfate produced greater potassium deficiency than did DOCA plus NaCl. Banks and Schwartz (10) found that the rate of titratable acid excretion was greater with infusions of polyvalent anions than with chloride. Recently, in experiments in our laboratory (14), Na₂SO₄ was shown to be more effective in promoting the secretion of H⁺ against pH gradients than was NaCl. Since in all of these experiments roughly equivalent amounts of Na⁺ were traversing the distal tubule, the presence of poorly reabsorbable anions must have exerted some additional influence on the secretion of both K⁺ and H⁺.

The increase in distal \( E_T \), demonstrated in the present studies, could readily explain the augmented K⁺ and H⁺ secretion produced by poorly reabsorbed anions. At least three possibilities, depending on the nature of the transport process, could explain the data. First, if K⁺ and H⁺ secretion is accomplished by a non-ion exchange process, such as a redox pump mechanism as proposed by Conway (15), then net secretion would be augmented by higher \( E_T \). If, however, K⁺ and H⁺ are secreted into the distal tubule lumen in exchange for tubular Na⁺, the higher \( E_T \), although having no direct effect on the pump mechanism, might restrain the back-diffusion of these cations and consequently augment net secretion. Finally, a third possibility is that K⁺ and H⁺ are passively distributed between distal tubular cell and luminal fluid. In such a system the higher the distal \( E_T \) the greater would be the rate of secretion. Although it is not possible on the basis of present evidence to identify which of the three mechanisms actually operate, the influence of a higher \( E_T \) would be to augment net K⁺ and H⁺ secretion in all instances.

It should not be construed from these remarks that the maximum K⁺ and H⁺ concentrations observed in bladder urine are entirely the consequence of distal

<table>
<thead>
<tr>
<th>Rat No.</th>
<th>Before Na₂SO₄</th>
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<tr>
<td>Mean</td>
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tubular activity. It is appreciated that the collecting tubule contributes to maximal urinary concentrations of these cations, both by water extraction as well as by secretion (16, 17). Nevertheless, it seems likely that the bulk of $K^+$ and $H^+$ secretion transpires in the distal tubule, and, moreover, it is conceivable that the effect of nonreabsorbable anions on $E_T$ also occurs in the collecting duct.

REFERENCES