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# Effect of Amiloride, Furosemide, and Ethacrynic Acid on Na Transport in the Rat Kidney[1] (38001)

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Studies on rats (1, 2) and dogs (1) have shown that amiloride (3,5-diamino-6-chloropyrazinoyl-guanidine induces natriuresis accompanied by a potassium sparing effect. These findings, plus the fact that Baer *et al.* (1) found an inhibition of sodium reabsorption in the distal samples, using the stop-flow technique, led to the conclusion that the site of action of amiloride is in the distal tubule.

Further studies have shown that the drug is a potent inhibitor of sodium transport in the amphibian skin and bladder (3, 4). In view of the latter, we decided to study the effect of amiloride on the proximal tubule of the rat using the microperfusion technique and compare its action to that of other diuretics.

*Methods.* Sprague–Dawley rats weighing 190 ± g were anesthetized with Inactin (100 mg/kg body wt) and then infused through the right jugular vein with Ringer's solution at a rate of 30 ml/hr-kg. Using the microperfusion technique and apparatus of Sonnenberg and Deetjen (5), proximal convoluted tubules were perfused at a rate of 20 nl/min with Ringer's solution containing $^{22}Na$ and $^{3}H$-inulin.

After attaining a control perfusion and concomitant collection of urine from approximately 1–3 separate proximal tubules in any given rat, each of the rats was injected through the left jugular vein with either amiloride (2 or 10 mg/kg body wt), furosemide (5 or 50 mg/kg body wt), or ethacrynic acid (5 or 10 mg/kg body wt). In each respective instance the level of diuretic was maintained by a constant infusion of amiloride (2 or 10 mg/hr-kg), furosemide (5 or 50 mg/hr-kg), or ethacrynic acid (5 or 10 mg/hr-kg). This was accomplished by dissolving the respective diuretic in Ringer's solution and maintaining the Ringer infusion rate of 30 ml/hr-kg. This rate was appropriately corrected for changes in urine flow. Under this diuretic condition, several proximal tubules were then perfused and urine samples collected concomitantly. Each of the tubules perfused in the control period and in the diuretic period was injected with a latex solution (6) for later tubule identification and measurement. Concentrations of $^{3}H$-inulin and $^{22}Na$ were determined with a three-channel liquid scintillation counter.

*Results. Control.* Thirty-eight proximal tubules were perfused in rats preceding administration of the diuretic. Figure 1 presents a semilogarithmic plot of collected-fluid to perfused-fluid concentration ratios ($C_{l(x)}/C_i$) of $^{3}H$-inulin and $^{22}Na$ versus the length of the perfused tubule. Correlation between the logarithm of the concentration ratios and perfused length was statistically significant ($P < 0.05$) for both $^{3}H$-inulin and $^{22}Na$. While the concentration of inulin increased, the concentration of $^{22}Na$ decreased along the perfused tubule. The slopes were $6.50 \times 10^{-2}$ mm$^{-1}$ and $-31.0 \times 10^{-2}$ mm$^{-1}$ for $^{3}H$-inulin and $^{22}Na$ respectively. That is, for each mm of tubule

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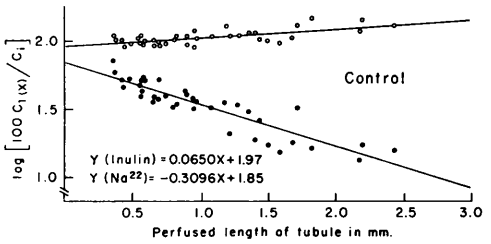


FIG. 1. Proximal tubular reabsorption of sodium. $C_{l(x)}$ is the concentration in collected sample; $C_i$ is the concentration in perfused fluid; (○) $^3$H-inulin; (●) $^{22}$Na. Lines are the calculated regression lines.

length, the $^3$H-inulin concentration increased by about 16%, and the $^{22}$Na concentration decreased by 50%.

Since $I_iV_i = I_lV_l$, where $I$ and $V$ are inulin concentration and tubule flow respectively, and subscripts $i$ and $l$ are at a distance 0 and 1 mm respectively, and since $100 - (100\ V_l/V_i) = R_{H_2O}$, the percentage reabsorption of water in 1-mm length, then the percentage reabsorption of water may be estimated from $R_{H_2O} = 100 - (100\ I_i/I_l)$. From the rate of increase in inulin, it follows that 14% of water was reabsorbed in 1 mm of tubule length.

From the percentage reabsorption rate of water, the perfusion rate ($20 \times 10^{-6}$ ml/min), and the concentration of Na in the perfusate (150 m$M$), an estimate of the net water and net sodium reabsorption may be obtained, assuming that the concentration of Na does not change along the proximal tubule. Values are presented in Table I. In order to obtain the reabsorptive values per unit area, the value of $10^{-3}$ cm was used as the radius for the proximal tubule. Also presented in Table I are the values for the unidirectional Na reabsorption, which are obtained from the slope of the $^{22}$Na regression line, making the appropriate correction for the $H_2O$ reabsorption, that is, change in $^3$H-inulin concentration. The unidirectional reabsorption flux of sodium, in control perfusions obtained as shown below, was at least 4 times greater than its net flux.

Data on various doses of each diuretic are lumped together since no appreciable dose effect was found.

*Amiloride.* Twenty-two proximal tubules were perfused in rats treated with amiloride, 2 mg/kg body wt, and 22 tubules in rats treated with amiloride, 10 mg/kg body wt. A plot of the data carried out as in the control experiments is presented in Fig. 2 (upper panel). The slopes of the regression lines are positive for $^3$H-inulin and negative for $^{22}$Na.

The slope of the regression line for $^3$H-inulin ($4.46 \times 10^{-2}$ mm$^{-1}$) is significantly different from zero ($P < 0.05$) and it is not significantly different from the $^3$H-inulin slope of the control group ($6.50 \times 10^{-2}$ mm$^{-1}$); on the other hand, the slope of the regression line for $^{22}$Na is not significantly different from zero and it was significantly different from the $^{22}$Na slope of the control group ($P < 0.05$).

As it may be seen in Table I, the estimated net reabsorption of water and Na in amiloride experiments were only slightly lower than or perhaps the same as in control experiments. On the other hand, the

TABLE I. Effect of Diuretics on Water and Sodium Reabsorption in the Proximal Tubule.[a]

| | $H_2O$ Reabsorption | Sodium reabsorption | |
|---|---|---|---|
| | Net | Unidirectional | Net |
| | $\mu$l/min-cm$^2$ | $\mu$moles/min-cm$^2$ | |
| Control (38) | 4.46 | 2.76 | 0.669 |
| Amiloride (43) | 3.18 | 1.78 | 0.478 |
| Furosemide (38) | 0.66 | 2.12 | 0.098 |
| Ethacrynic acid (35) | 4.89 | 2.80 | 0.733 |

[a] Values are obtained from the regression lines of Figs. 1 and 2. Various doses of each diuretic have been lumped into one group. Number of samples in parentheses.

unidirectional Na reabsorption in amiloride was 64% of the control experiments.

*Furosemide.* Figure 2 (middle panel) presents a plot of the data obtained from 18 proximal tubules perfused in rats treated with 5 mg/kg body wt and from 21 tubules in rats treated with 50 mg/kg body wt of furosemide.

The slope of the regression line for $^{3}$H-inulin is not significantly different from zero, while it is significantly different from that obtained in control perfusions ($P < 0.05$). The slope of the regression line for $^{22}$Na is not significantly different from zero, while it is significantly different from the slope of the control group ($P < 0.05$).

The estimated net reabsorption of water and Na in furosemide-treated rats (Table I) was 15% of the control experiments. The unidirectional flux was 77% of the control experiments. That is, furosemide reduced markedly the net, while only slightly the unidirectional, reabsorption of Na.

*Ethacrynic acid.* The lower panel of Fig. 2 presents a plot of the data obtained from 14 proximal tubules in rats treated with ethacrynic acid 5 mg/kg body wt and from 21 tubules in rats treated with ethacrynic acid, 50 mg/kg body wt.

The slopes of the regression lines for both $^{3}$H-inulin and $^{22}$Na are essentially the same as the slopes of control perfusions. The only statistical parameter which differs from control perfusions is that the slopes for $^{22}$Na are not statistically different from zero whether the two groups are studied separately or both in one group.

The estimated net reabsorption of water and Na (Table I) and unidirectional reabsorption of Na was about the same in ethacrynic acid as in control experiments.

*Effect of diuretics on the unidirectional transtubular permeability of $^{22}$Na.* Since a change in concentration of $^{3}$H-inulin occurred in all experiments, although the change was statistically not significant in furosemide experiments, the following equation has been used to estimate the $^{22}$Na permeability:

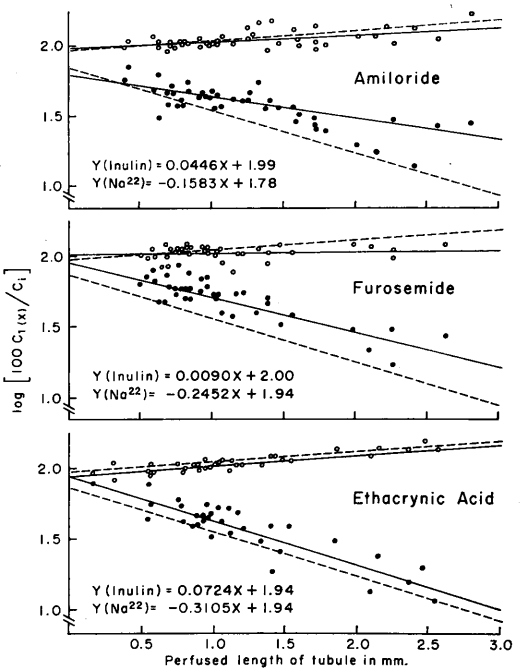


FIG. 2. Effect of diuretics on proximal tubular reabsorption of sodium. Symbols are the same as in Fig. 1. Continuous lines are the calculated regression lines under diuretic influence. Broken lines are the control regression lines from Fig. 1.

where $P_{1,2}$ is the transtubular permeability; $V_i$ is the perfusion rate; $r_l$ is the radius of the tubule; $x$ is the length of the perfused tubule; $I_i$ is the concentration of inulin in the perfusate; $I_{l(x)}$ is the concentration of inulin at point $x$; $C_i$ is the concentration of $^{22}$Na in the perfusate; and $C_{l(x)}$ is the concentration of $^{22}$Na at point $x$.

This equation may be used when there is movement of water and therefore was adapted by us (7) from Curran and Solomon (8). It is analogous to the equation used by Grantham and Burg (9) and Morgan and Berliner (10).

The estimated permeabilities are in Table II. The two-dose subgroups in each diuretic have been placed in one group because there was not much difference between the permeabilities of each subgroup.

The unidirectional permeability of so-

$$P_{1,2} = \frac{V_i}{2\pi r_l x}\left[1 - \frac{I_i}{I_{i(x)}}\right] \cdot \left[\frac{\log [C_{l(x)}/C_i]}{\log [I_i/I_{l(x)}]} + 1\right] \qquad (1)$$

TABLE II. Effect of Diuretics on the Unidirectional Permeability of Na in the Proximal Tubule.[a]

| Control | Amiloride | Furosemide cm/sec × $10^5$ | Ethacrynic acid |
|---|---|---|---|
| 62.6 ± 2.3 (38) | 46.7 ± 2.0 (43) | 36.8 ± 1.7 (38) | 44.5 ± 1.6 (35) |

[a] Values are means ± SEM and number of samples. Various doses of each diuretic have been lumped into one group.

dium of each diuretic group was significantly lower than that of the control group ($P < 0.01$). The permeability of the furosemide group was lower than that of the amiloride or ethacrynic acid groups ($P < 0.01$). No difference in permeability was found between the amiloride and ethacrynic acid groups. The higher dose subgroups had a lower permeability to sodium than the lower dose subgroups: 10% for amiloride, 13% for furosemide, and 2% for ethacrynic acid. These differences were not statistically significant.

*Discussion.* The net reabsorption of water and therefore of sodium salts was markedly inhibited by furosemide, while no (or a negligible) effect was found on this parameter by amiloride, and no effect at all by ethacrynic acid.

On the other hand, both furosemide and amiloride had an inhibitory effect on the unidirectional movement of Na across the proximal tubule wall. The inhibitory effect was greater for furosemide whether it was studied for the overall group from the slopes of the regression lines (Table I) or whether the movement of sodium was studied by Eq. (1), in which case the permeability was calculated for each sample independently (Table II). The effect of amiloride on the unidirectional $^{22}$Na reabsorption was significant ($P < 0.05$) when estimated by either of the two methods (Table I or II).

In the case of ethacrynic acid, no effect was found on the unidirectional sodium reabsorption, with the regression line method (Table I), while the permeability was significantly lower for the ethacrynic acid group than for the control group ($P < 0.05$) when Eq. (1) was used (Table II). There is a sound mathematical reason for this apparent discrepancy: while the slopes of the regression lines for Na in the ethacrynic acid and control groups were equal, the absolute value of the intercept of the control group was smaller than the intercept of the ethacrynic acid group. That is, at any point in the abscissa, the absolute value of the ordinate was smaller for the ethacrynic acid than for the control group. Perhaps this means that ethacrynic acid has an inhibitory effect on the unidirectional Na reabsorption in the proximal tubule; nevertheless, we would prefer to reach this conclusion with more solid data.

How could one explain the fact that amiloride had an inhibitory effect on the unidirectional $^{22}$Na movement without affecting the net reabsorption of water? This could be easily explained if one assumes that the movement of Na between the lumen and the interstitium takes place through two pathways, one intracellular and one intercellular, as shown by Windhager *et al.* and Giebisch *et al.* (11, 12). In the intracellular pathway, Na enters the cell from the lumen (luminal membrane) by passive transport while Na is actively pumped from the cell into the interstitium (peritubular membrane). The intercellular movement is of a passive nature. Our data suggest that when amiloride is present in the blood and not in the perfusate, it does not affect the intracellular pathway since an inhibition in either the luminal or peritubular membrane should result in a decrease in the net reabsorption of Na and therefore of water, which did not occur. The decrease in the unidirectional flux of $^{22}$Na can be explained by a decrease in the permeability to Na in the intercellular pathway. Furthermore, as pointed out by Giebisch, Boulpaep, and Whittembury (12),

the cation movement is much higher than the anion movement, apparently due to the presence of negative charges in the tight junction. Therefore, an inhibitor (e.g., amiloride) could affect the bidirectional movement of Na (or unidirectional movement of $^{22}Na$) without noticeably affecting its net movement as well as the net movement of water or other substances.

The inhibitory action of furosemide must have been on the intracellular pathway since it resulted in a decrease not only in the unidirectional movement of $^{22}Na$ but also in a decrease in the net water reabsorption and, therefore, in the net Na reabsorption. These data go along with those of Morgan *et al.* (13) who found that furosemide decreased the net reabsorption of water in the proximal tubule by about 40%. Of interest is the fact that the effect of furosemide on the net sodium reabsorption was much greater than its effect on the unidirectional reabsorption of $^{22}Na$. These data suggest that the intracellular pathway constitutes a small fraction of the total transtubular flux of $^{22}Na$.

*Conclusion.* In conclusion, our data obtained by intraluminal perfusion of proximal tubules with rat Ringer's containing $^{22}Na$ and $^{3}H$-inulin, and with the presence of amiloride, furosemide, or ethacrynic acid in blood and not in the perfusate, can be easily explained if Na is reabsorbed via two parallel pathways: furosemide would inhibit the intracellular (active) pathway, amiloride would inhibit the intercellular (passive) pathway, and ethacrynic acid would not have any effect on either pathway.

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