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Effect of Intraluminal Amiloride on Na Transport in the Rat Proximal Tubule (38130)

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Amiloride (3,5-diamino-6-chloropyrazinoylguanidine) was found to inhibit the unidirectional flux of ^{22}Na in perfused proximal tubules of rats when the drug was present in the blood (1). The drug did not inhibit the net water reabsorption, therefore, it apparently did not have an affect on the active reabsorption of sodium.

Since amiloride has been found to be a potent inhibitor of sodium reabsorption in the amphibian bladder and skin (2, 3) and since it has been found by the same authors that the inhibition of sodium transport is about one thousand times higher when the drug is on the source than when it is on the sink site of the sodium transport, experiments were designed to test the effect of the drug when present in the luminal and not in the interstitial site of the tubule cell.

Methods. Sprague-Dawley rats weighing about 200 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 a modification of the microperfusion technique and apparatus of Sonnenberg and Deetjen (4), proximal tubules were perfused at a rate of 20 nanoliters per min.

Two pumps were used, one contained a control solution (Ringer's with ^{22}Na and ^{14}C-inulin) and the other contained the same solution plus 10^{-4} *M* amiloride. A selected proximal tubule was first perfused with the control solution for 30–40 min, and 2–4 samples were obtained during this period. Then the control pump was withdrawn and substituted by the "amiloride" pump. Perfusion was restarted at exactly the same location. The amiloride perfusion was administered for 45–60 min during which time 2–4 samples were obtained. The use of the 2 pumps was randomized with reference to their use for control or for amiloride perfusions.

Each of the tubules perfused in the control period and in the diuretic period was injected with a latex solution (5) for later tubule identification and measurement. Concentrations of ^{14}C-inulin and ^{22}Na were determined with a three channel liquid scintillation counter.

Results. Figure 1, presents a plot of the concentration ratio of collected over perfused fluid [(TF)/(IF)] of ^{14}C-inulin and ^{22}Na, versus time. In this selected experiment three perfusion periods are presented: control, amiloride, and recovery or return to control. The (TF)/(IF) values for inulin were definitely reduced by amiloride and increased slightly when the amiloride perfusate was substituted by the control. The (TF)/(IF) values for sodium were definitely increased by amiloride returning to low levels when a control perfusate was reinstated.

Table I, presents compiled data from eight perfusions (8 rats). The values presented for

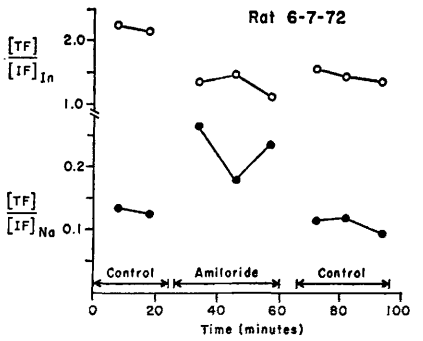

FIG. 1. Effect of amiloride on proximal tubular reabsorption of water and sodium. Open circles are the collected fluid to perfusate ratios ([TF]/[IF]) of inulin concentration; dots are the same ratios for Na. Zero time refers to the beginning of the tubular perfusion.

TABLE I. Effect of 10^{-4} M Amiloride on Water Reabsorption and Na Permeability.[a]

Rat	Tubule length (mm)	[TF]/[IF]				^{22}Na Permeability ([cm/sec] × 10^5)		
		^{14}C-inulin		^{22}Na				
		Control	Amil.	Control	Amil.	Control	Amil.	% Change
A	1.31	1.16 (2)	1.02 (3)	0.43	0.41	37.7	37.1	− 1.6
B	0.71	1.14 (2)	1.05 (3)	0.19	0.17	128.0	133.0	+ 3.9
C	0.89	1.22 (2)	0.94 (1)	0.45	0.48	43.0	33.3	− 22.6
D	2.24	2.22 (2)	1.30 (3)	0.13	0.22	37.0	29.3	− 20.8
E	2.95	1.32 (3)	0.88 (1)	0.11	0.15	35.9	31.2	− 13.1
F	0.94	2.44 (2)	0.91 (3)	0.20	0.30	93.0	50.8	− 45.5
G	0.82	2.19 (2)	0.89 (3)	0.12	0.31	106.4	58.0	− 45.5
H	0.94	1.34 (2)	0.85 (3)	0.27	0.43	62.2	33.0	− 46.9
Mean		1.63	0.98	0.24	0.32	67.9	50.7	− 24.0
Sem		± 0.19	± 0.05	± 0.05	± 0.04	± 12.9	± 12.3	± 7.1

[a] [TF]/[IF] = concentration ration of collected perfused solutions. Values are the mean of the 1–3 samples obtained during the control or amiloride period (amil.). Values within the parentheses are number of samples during the given period.

the (TF)/(IF) ratios and the ^{22}Na permeability are the mean values of the samples collected during the control period and the mean values of the samples collected during the amiloride period.

Amiloride uniformly and significantly decreased the (TF)/(IF) values of ^{14}C-inulin ($P < 0.02$). The increase in the (TF)/(IF) values of ^{22}Na was not uniform but it was statistically significant ($P < 0.05$).

Values on ^{22}Na permeability presented in the three columns on the right side of the table were obtained using the following equation,

$$P_{1,2} = \frac{V_i}{2\pi r_1 x}\left[1 - \frac{I_i}{I_{1(x)}}\right] \cdot \left[\frac{\log [C_{1(x)}/C_i]}{\log [I_i/I_{1(x)}]} + 1\right], \quad (1)$$

where $P_{1,2}$ is the transtubular permeability; V_i is the perfusion rate; r_1 is the radius of the tubule; x is the length of the perfused tubule; I_i is the concentration of inulin in the perfusate; $I_{1(x)}$ is the concentration of inulin at point x; C_i is the concentration of ^{22}Na in the perfusate; and $C_{1(x)}$ 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 (6), from Curran and Solomon (7). It is analogous to the equation used by Grantham and Burg (8), and Morgan and Berliner (9).

Amiloride decreased the unidirectional permeability of ^{22}Na by about 24%. Although the effect was not uniform it was statistically significant ($P < 0.01$). The only 2 experiments which had an inhibitory effect by amiloride of less than 10% were those which had (TF)/(IF) inulin ratios lower than 1.20 in the control period (rats A and B).

Discussion. From present experiments it is apparent that amiloride, when present in the lumen of the proximal tubule in concentrations of 10^{-4} *M*, completely inhibits the net reabsorption of water. Since water reabsorption depends mostly on the net or active transport of Na in the proximal tubule, the data suggest that amiloride inhibits the active sodium transport. This is contrary to what we observed previously (1), when amiloride was present on the blood site only during perfusion of the tubule. In that situation amiloride

inhibited only the unidirectional flux of ^{22}Na without having any effect on the net water reabsorption. We attributed the effect from the blood on an inhibitory action on the passive movement of sodium through the tight junction, that is, an effect in the intercellular or parallel pathway of Giebisch, Boulpaep and Whithembury (10).

Whether the effect of amiloride when present in the lumen is on the active pump (peritubular membrane) (10) or on the passive entrance from the lumen into the cell we cannot tell from these experiments. Nevertheless, since the effect of amiloride is so different when present in the blood site only, a strong possibility exists that amiloride may inhibit the passive entrance of sodium into the cell as it has been shown to occur in the toad urinary bladder (2) and frog skin (3).

Furthermore, in view of the fact that the net reabsorption of Na is absolutely abolished by amiloride when present in the perfusate while it is not affected when administered systemically, and in view of the fact that in both instances the unidirectional ^{22}Na permeabilities were affected to the same extent (Table II, Ref. 1 and compare to Table I of the present paper), it is strongly suggestive that the unidirectional fluxes of Na through the intercellular pathway are much greater than the net movement of Na.

In conclusion, amiloride when present in the lumen of the proximal tubule of the rat in concentrations 10^{-4} *M*, strongly inhibits the net reabsorption of Na as demonstrated by the inhibition of water reabsorption. The inhibition of the unidirectional Na flux appears to be equally affected when amiloride is only in the lumen or only in the interstitial site of the tubule.

1. Wilczewski, T.W., Olson, A.K., and Carrasquer, G., Proc. Soc. Exp. Biol. and Med., in press, April (1974).

2. Bentley, P.J., J. Physiol. **195**, 317 (1968).

3. Ehrlich, E.N. and Crabbe, J., Pflugers Arch. Ges. Physiol. **302**, 79 (1968).

4. Sonnenberg, H., and Deetjen, P., Pflugers Arch. **278**, 669 (1964).

5. Malnic, G., Klose, R.M., Giebisch, G., Amer. J. Physiol. **206**, 674 (1964).

6. Wilczewski, T.W., Sonnenberg, H., Carrasquer, G., Proc. Soc. Exp. Biol. and Med., **135**, 609 (1970).

7. Curran, P.F., Solomon, A.K., J. Gen. Physiol. **41**, 143 (1957).

8. Grantham, J.J., Burg, M.B., Amer. J. Physiol. **211**, 255 (1966).

9. Morgan, T., Berliner, R.W., Amer. J. Physiol. **215**, 108 (1968).

10. Giebisch, G., Boulpaep, E.L., Whittembury, G., Phil. Trans. Roy. Soc. Lond. B. **262**, 175 (1971).

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