Effects of Amiloride on the *in Vitro* Frog Gastric Mucosa (37614)

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Amiloride, a pyrazine diuretic, has been shown to decrease the secretion of K^+ by the renal tubule (1–3). Although this effect has been explained as a result of the inhibitory action of the drug on Na^+ reabsorption, the possibility exists that amiloride might directly affect K^+ permeability. This thought led to the speculation that amiloride might likewise affect the K^+ permeability of the frog gastric mucosa which lends itself well to such an investigation. From the literature (4, 5), it appears that the K^+ resistance of the nutrient membrane of frog gastric mucosa is about half the Cl^- resistance and that other ionic resistances are very high relative to the K^+ and Cl^- resistances.

Since K^+ has been found to be essential for HCl secretion (6), perhaps if amiloride affected the K^+ permeability it might also decrease the H^+ secretory rate. Experiments were, therefore, designed to test the effect of amiloride on the transmembrane resistance, the transmembrane potential difference (PD), and the H^+ secretory rate in high and low nutrient K^+-solutions.

Methods. The experiments were performed on gastric mucosae of *Rana pipiens* with an *in vitro* method described in detail elsewhere (7). In this method each mucosa was mounted between cylindrical chambers. Two pairs of electrodes were used, one for sending current across the mucosa and the other for measuring the PD. The resistance was determined as the change in PD per unit of applied current. The nutrient bathing solution contained (m*M*): Na^+, 101; K^+, 4; Ca^{2+}, 1; Mg^{2+}, 0.8; Cl^-, 81; HCO_3^-, 25; phosphate, 1.0; and glucose, 10; and the secretory bathing solution: Na^+, 102; K^+, 4; Cl^-, 106. In high K^+ nutrient solutions, K^+ replaced Na^+. Both sides of the mucosa were gassed with 95% O_2 and 5% CO_2. The pH of the secretory solution was maintained at 4.90. After the control part of the experiment, amiloride was added in the nutrient solution to a 0.5 or 2.0 m*M* concentration.

Results. Figure 1 shows the effects of adding amiloride to the nutrient solution. At the time indicated by the arrow, amiloride was added to a concentration of 0.5 m*M* in the nutrient solution. A comparison was generally made of the changes in the parameters in about 30 min following the addition of amiloride in the nutrient solution to the control values just before the addition. As shown in Fig. 1, the resistance increased about 50%, the PD decreased about 15%, and the H^+ rate decreased about 30%. Washing both sides of the mucosa with regular solutions produced only a partial recovery of the parameters towards control values. In about 40% of the experiments after washing with regular solutions, the resistance decreased to some extent towards control values while the H^+ rate persisted at about the same level as with amiloride in the nutrient solution.

Table I summarizes the main results. Columns III, V and VII give, respectively, the average resistances, PD and H^+ rates of mucosae bathed in regular solutions without amiloride. Columns IV, VI and VIII give, respectively, the average percentage changes of the resistance, PD and H^+ rate following the addition of amiloride to the nutrient solution. In the 4 m*M* K^+ nutrient solution, both 0.5 and 2.0 m*M* amiloride induced the changes shown in Fig. 1 and summarized in Table I, that is, the resistance increased, the PD decreased and the H^+ secretory rate decreased. Furthermore, the increase in resistance induced by 2.0 m*M*

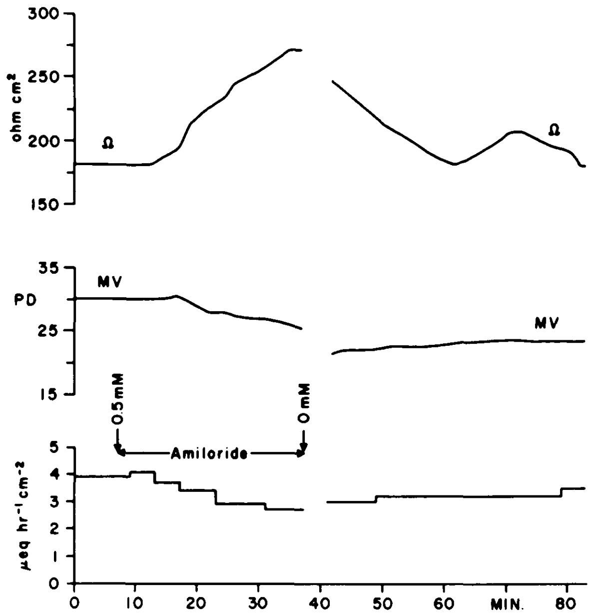


FIG. 1. The effects of amiloride on the resistance, PD and H^+ secretory rate of frog gastric mucosa in chloride solutions. At the time indicated by the first arrow, amiloride was added to a concentration of 0.5 m*M* in the nutrient solution and at the time indicated by the second arrow, the amiloride was removed. Resistance, PD, and H^+ secretory rate are plotted versus time.

amiloride was significantly higher than the increase induced by 0.5 m*M* amiloride ($p < 0.05$). In the 79 m*M* K^+ nutrient solution, both 0.5 m*M* amiloride and 2.0 m*M* amiloride increased the resistance and decreased the H^+ secretory rate. The PD, however, with 0.5 m*M* amiloride did not change significantly while with 2.0 m*M* amiloride the PD increased. As in the case of 4 m*M* K^+, the increase in amiloride from 0.5 to 2.0 m*M* in the 79 m*M* K^+ nutrient solution caused a significant increase in resistance ($p < 0.01$). In all cases the H^+ rate decreased by approximately the same amount.

TABLE I. Effects of Amiloride on Resistance, PD, and H^+ Secretory Rate of Frog Gastric Mucosa.

No. of expts	Description	R (Ω cm²)	$\Delta R/R$ (%)	PD (mV)	ΔPD/PD (%)	$\dot{H}$[a] (μEq hr⁻¹ cm⁻²)	$\Delta\dot{H}/\dot{H}$ (%)
7	4 m*M* K^+ 0.5 m*M* Amiloride	240 ± 95	36 ± 18 ($p < 0.01$)	18 ± 4[b]	-24 ± 8 ($p < 0.01$)	4.1 ± 0.8	-19 ± 11[c] ($p < 0.01$)
6	4 m*M* K^+ 2.0 m*M* Amiloride	238 ± 46	150 ± 108 ($p < 0.02$)	26 ± 6	-24 ± 12 ($p < 0.01$)	4.5 ± 0.8	-27 ± 17 ($p < 0.02$)
8	79 m*M* K^+ 0.5 m*M* Amiloride	128 ± 20	11 ± 9 ($p < 0.01$)	-18 ± 5	8 ± 12 ($p > 0.10$)	2.6 ± 0.9	-21 ± 8 ($p < 0.01$)
6	79 m*M* K^+ 2 m*M* Amiloride	120 ± 41	82 ± 36 ($p < 0.01$)	-22 ± 2	20 ± 9.5 ($p < 0.01$)	3.4 ± 1.0	-25 ± 7 ($p < 0.01$)

[a] $\dot{H}$ represents H^+ secretory rate.

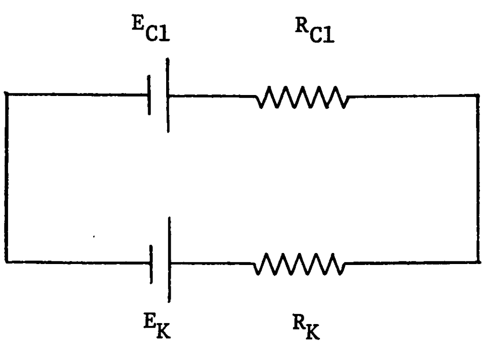
[b] Positive PD implies that the serosal (nutrient) side is positive relative to the mucosal (secretory) side.

[c] In each case the number following the $\pm$ sign denotes the standard deviation.

In two experiments amiloride was added to the secretory side to a concentration of 1 m*M* in a regular secretory solution with the nutrient side containing 4 m*M* K^+. No significant effects on the electrophysiological parameters were observed.

Discussion. Agents or procedures which markedly increase the resistance produce a concurrent marked decrease in the H^+ secretory rate (8, 9). This finding can be explained on the assumption of an electrogenic mechanism for HCl secretion in the frog gastric mucosa (7). Amiloride like barium (10) produced relatively low changes in H^+ rate for relatively high changes in resistance. It appears, therefore, that the site of action of amiloride is not the secretory membrane where the electrogenic pumps are presumed to be located but rather the nutrient membrane. In terms of this latter hypothesis, the results can be readily explained.

Figure 2 depicts the K^+ and Cl^- pathways across the nutrient membrane which are the only low resistance pathways of this membrane (5, 11). Figure 2 also shows two equations, each representing the PD across the nutrient membrane from the nutrient solution to the cell. In equilibrium $E_{Cl} = E_K$



$$E_{Cl} > E_K$$

$$PD = E_{Cl} - \frac{(E_{Cl} - E_K)R_{Cl}}{R_{Cl} + R_K}$$

$$PD = E_K + \frac{(E_{Cl} - E_K)R_K}{R_{Cl} + R_K}$$

FIG. 2. Electrical circuit of nutrient membrane showing the K^+ and Cl^- resistance pathways.

where E_{Cl} represents the emf due to ion gradients across the Cl^- pathway and E_K the emf across the K^+ pathway (5, 6). The equilibrium cellular concentrations of "free" K^+ and Cl^- are, respectively, 8 and 40 m*M* (5). However, since Cl^- is pumped from the cell across the secretory membrane and K^+ accompanies Cl^- to some extent, in general we would expect $E_{Cl} > E_K$.

In terms of the equations in Fig. 2, the changes in PD can be explained. If we assume that the effect of amiloride is primarily an increase of the resistance of the K^+ pathway, then from the first equation, the PD after amiloride would yield a larger PD. In the second term on the right, E_{Cl} being greater than E_K means that $E_{Cl} - E_K$ is positive. Also, since R_K in this term occurs in the denominator, any increase in R_K would decrease this term which subtracted from the first term would yield a higher PD value. If we assume the effect is primarily an increase of the resistance of the Cl^- pathway, then from the second equation by similar reasoning, the PD would decrease after amiloride. We can infer then that for 4 m*M* K^+, the main effect of amiloride is an increase in the resistance of the Cl^- pathway. The resistance of the K^+ pathway may be affected but to a lesser extent.

For 79 m*M* K^+, the emf's are both reversed and now $E_K > E_{Cl}$. For purposes of discussion, we assume that there are 80 m*M* K^+ and 80 m*M* Cl^- in the nutrient solution with a "product constant" (5) $= 6400$. If this product constant holds in the cell, we obtain cellular concentrations of 66 m*M* K^+ and 98 m*M* Cl^- which account for the reversed polarity. Similar arguments to the above indicate that, with 79 m*M* K^+ and a reduced resistance, therefore, of the nutrient membrane, the effect of 0.5 m*M* amiloride may influence either the K^+ or Cl^- pathway. However, 2.0 m*M* amiloride increases predominantly the resistance of the K^+ pathway.

Calculations based on $R_{Cl} = 2\ R_K$ and reasonable assumptions of the resistance of the secretory membrane (11) lead to the same results. Amiloride affects both the K^+ and Cl^- permeability of the nutrient membrane. For low K^+, the effect of amiloride is predominantly a decrease of Cl^- permeability and, for high K^+ with sufficient amiloride, the effect is predominantly a decrease of K^+ permeability.

Since K^+ is essential for acid secretion (6) and Cl^- is electrically and biochemically coupled to H^+ (7), any effect on K^+ and Cl^- permeability would be expected to reflect itself in a change of the H^+ secretory rate.

Conclusions. Concentrations of 2 m*M* amiloride in the nutrient solution resulted in a marked increase in resistance and relatively little change in the H^+ secretory rate. The PD data coupled to these results suggested that amiloride decreases both K^+ and Cl^- permeability of the nutrient membrane. In low K^+ experiments the effect predominates on the Cl^- pathway whereas in high K^+ the effect predominates on the K^+ pathway.

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