Biochimica et Biophysica Acta, 509 (1978) 390—394

BBA Report

BBA 71341

AMILORIDE-INDUCED STIMULATION OF HCO_3^- REABSORPTION IN TURTLE BLADDER

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(Received November 21st, 1977)

Summary

Amiloride in the mucosal fluid (at concentrations of $5 \cdot 10^{-6}$ M to 10^{-4} M) reversibly stimulates the HCO_3^--dependent moiety of the short-circuiting current (I_{sc}) in ouabain-treated turtle bladders bathed by Na-free Ringer solutions with or without Cl^-.
This effect is uniquely different from the known inhibitory effect of this agent on Na^+ transport. Thus, any comprehensive hypothesis on the action of amiloride over a wide dosage-response range should take into account its effect on HCO_3^- transport.

During a study of the possible adrenergic control of anion transport in turtle bladders bathed by Na-free Ringer solutions, the prior mucosal addition of amiloride (10^{-5} M to 10^{-4} M) apparently prevented the previously reported [1, 2] stimulation of the anion-related short-circuiting current by the mucosal addition of norepinephrine (10^{-5} M to 10^{-4} M). It was then found that the mucosal addition of amiloride alone produced a stimulation of this anion-related short-circuiting current across bladders in Na-free Ringer media. The present report deals with some characteristics of this amiloride-induced increase in anion reabsorption.

Procedures and methods for evaluating transepithelial potential (*PD*), short-circuiting current (I_{sc}), dc-resistance (R), and $^{36}Cl^-$ fluxes have been described [3, 4]. Bladders of *Pseudemys scripta* turtles were bathed on both surfaces by Na-free choline Ringer solutions plus 10^{-4} M ouabain (Sigma) in

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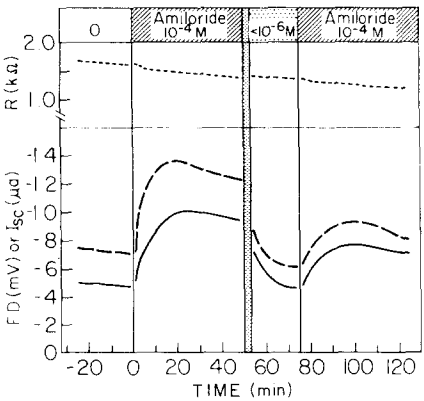

Fig. 1. Effect of 10^{-4} M amiloride on *PD*, I_{sc}, and *R* of ouabain-treated bladders in choline (Cl^- + HCO_3^-) bathing system. Experimental conditions are described under Table I. *R* is depicted by dotted line in upper panel; *PD* is depicted by the dashed line and I_{sc} by the solid line in lower panel. Negative values of *PD* and I_{sc} denote transport of negative charges from M-to-S. Width of shaded vertical column denotes time required for reducing mucosal amiloride concentration by at least 6 sequential replacements of ½ the chamber volume (10 ml) with fresh bathing medium. This serial dilution procedure does not introduce the mechanical perturbations which in a complete replacement and rinsing of the mucosal compartment decrease transepithelial resistance. The magnitude of the second stimulation of *PD* and I_{sc} by 10^{-4} M amiloride was about 60—70% of the first response.

the serosal fluid to inhibit any residual Na transport [5]. In some experiments Na-rich Ringer solutions plus ouabain were used.

Fig. 1 shows the effect of amiloride on a ouabain-treated bladder bathed on both surfaces by an identical Na-free, (Cl^- + HCO_3^-)-containing choline solution. The *PD* and I_{sc} increased within 0.5 min after the first addition of amiloride (10^{-4} M) to the mucosal fluid and reached a maximal value in about 20 min. Replacement of mucosal fluid by fresh Ringer solution restored the anion transport parameters to control levels. A second addition of amiloride to mucosal fluid resulted in a second increase in *PD* and I_{sc}.

Other results were the following: (i) amiloride in mucosal fluid stimulated the *PD* and I_{sc} in ouabain-treated bladders bathed by Na-rich ($N = 3$) as well as the Na-free solutions; (ii) the threshold, half-maximal, and maximal responses to amiloride occurred at concentrations of 10^{-5} M, $3 \cdot 10^{-5}$ M, and 10^{-4} M respectively; (iii) amiloride (10^{-3} M) in serosal fluid failed to stimulate the *PD* or I_{sc} ($N = 2$); (iv) mucosal amiloride (10^{-4} M) failed to increase *PD* or I_{sc}, when these parameters were first stimulated by mucosal norepinephrine (10^{-4} M) ($N = 3$).

Table I shows that in 13 bladders bathed by the (Cl^- + HCO_3^-) solutions, amiloride induced significant increases in *PD* and I_{sc} and a small, but significant, decrease in transepithelial *R*. In three experiments on the mucosal to serosal (M-to-S) flux of $^{36}Cl^-$, the mean value of this flux was 10.2 ± 2.8 μA and remained unchanged (10.6 ± 2.9 μA) for 3 h after amiloride addition, whereas the I_{sc} was increased by 65 ± 9% in 20 min.

Since amiloride did not change the M-to-S flow of Cl^-, it can be assumed that the amiloride-induced increment in the I_{sc} is due solely to an increase in the pumping of HCO_3^- from M-to-S. If this assumption is correct, amiloride should increase the I_{sc} (and *PD*) in the presence of, but not in the

TABLE I

EFFECT OF AMILORIDE ON *PD*, I_{sc}, AND *R* OF BLADDERS IN Cl^--RICH, HCO_3^--RICH BATHING SYSTEM

Mean values ±S.E. (N = 13) of *PD*, I_{sc}, and *R* and of the percentage changes in these parameters at the time of maximal response after the mucosal addition of 10^{-4} M amiloride. Sign convention: (M) electropositive to (S). Maximal response after 22 ± 5 min. Area of exposed tissue, 1.5 cm^2. Composition of bathing fluid (mM): choline chloride, 21; choline bicarbonate, 20; choline sulfate, 30; KCl, 4; $MgSO_4$, 0.8; K_2HPO_4, 0.61; KH_2PO_4, 0.14; $CaSO_4$, 2; glucose, 11; sucrose, 20. Osmolality was 220 mosM/kg; final pH, 7.5 ± 0.1; gassed with H_2O-saturated 98% O_2/2% CO_2; ouabain, 10^{-4} M in (S).

Condition	*PD* (mV)	I_{sc} (μA)	*R* (kΩ)
Before amiloride	18.2 ± 3.8	11.0 ± 2.0	1.6 ± 0.1
After amiloride	28.7 ± 5.5	19.7 ± 3.0	1.4 ± 0.1
Δ(%)*	+76.8 ± 12.2	+94.1 ± 11.9	−9.9 ± 2.0
$P(\Delta=0)$	< 0.001	< 0.001	< 0.001

*Note that values of Δ(%) are means ±S.E. of N individual percentage changes in the designated electrical parameters after addition of amiloride. Statistical significance of Δ was calculated by the Student's *t*-test.

TABLE II

EFFECT OF AMILORIDE ON *PD*, I_{sc}, AND *R* OF BLADDERS BATHED BY HCO_3^--RICH, Cl^--FREE BATHING SYSTEM

Mean values ±S.E. (N = 9) of *PD*, I_{sc}, and *R* and of the percentage changes in these parameters after mucosal addition of 10^{-4} M amiloride. Composition of bathing fluid was similar to that described in Table I except that Cl^- was replaced by SO_4^{2-} without changing the choline and K^+ concentrations. Osmolality was readjusted with sucrose. Maximal response after 26 ± 6 min. Other experimental conditions and statistical definitions are given in Table I. Increases in values of *PD*, I_{sc} were not significantly different from those in Table I (P >0.05 and P >0.2, respectively)

Condition	*PD* (mV)	I_{sc} (μA)	*R* (kΩ)
Before amiloride	13.7 ± 2.8	8.3 ± 2.4	2.5 ± 0.5
After amiloride	29.0 ± 6.0	16.3 ± 4.6	2.4 ± 0.5
Δ(%)	118 ± 14	123 ± 25	−4.7 ± 4.1
$P(\Delta=0)$	<0.001	<0.001	>0.2

TABLE III

EFFECT OF AMILORIDE ON *PD*, I_{sc}, AND *R* OF BLADDERS BATHED BY HCO_3^--FREE, Cl^--RICH MUCOSAL BATHING SYSTEM

Mean values ±S.E. (N = 4) of *PD*, I_{sc}, and *R* and of the percentage changes in these parameters after mucosal addition of 10^{-4} M amiloride. Composition of serosal fluid is described in Table I. Composition of mucosal fluid was similar to the serosal fluid except that HCO_3^- was replaced by SO_4^{2-}. Osmolality was readjusted with sucrose; final pH, 7.6; gassed with H_2O-saturated 100% O_2. Other experimental conditions, and statistical definitions are given in Table I.

Condition	*PD* (mV)	I_{sc} (μA)	*R* (kΩ)
Before amiloride	10.1 ± 2.1	8.2 ± 0.8	1.3 ± 0.3
After amiloride	9.6 ± 2.4	7.0 ± 0.9	1.4 ± 0.3
Δ(%)	− 7.4 ± 11.4	−12.3 ± 15.9	−9.7 ± 5.9
$P(\Delta=0)$	>0.5	>0.4	>0.1

absence of HCO_3^- in mucosal fluid. This prediction was confirmed in the following experiments: First, with HCO_3^- but no Cl^- present in both mucosal and serosal fluid, the I_{sc} approximates the net reabsorption of HCO_3^- [4, 6]; under these bathing conditions, amiloride stimulated the I_{sc} (Table II). Secondly, with no exogenous HCO_3^- in the mucosal fluid (Cl^- present in mucosal, and Cl^- plus HCO_3^- present in serosal fluid), amiloride failed to stimulate the I_{sc} (Table III). Moreover, since under these bathing conditions the I_{sc} approximates the net Cl^- reabsorption [3], these data indicate a lack of effect by amiloride on Cl^- transport.

The data (Tables I–III) are completely accounted for by considering an amiloride-induced increase in the HCO_3^- moiety of I_{sc} and the hypothesis that the I_{sc} equals the algebraic sum of the conductive flows of Cl^- and HCO_3^- [4, 5, 7, 8]. An alternative hypothesis that the I_{sc} is due solely to the secretory pumping of protons and that all Cl reabsorption is non-conductive [9, 10] cannot account for the data. If the I_{sc} were generated by a proton secretion from cell to mucosal fluid, it should be stimulated by amiloride, whether or not HCO_3^- is present in the mucosal fluid. This is contrary to the facts (Table III).

Data from studies on the functional groups of amiloride (3,5-diamino-6-chloropyrazinoyl guanidine · HCl) suggest that the guanidine group is required for the stimulation of HCO_3^- transport as well as for the inhibition of Na^+ transport [11, 12, 13]. In 4 experiments, a guanidinium-free analogue (methyl-3, 5-diamino-6-chloropyrazinoate) failed to stimulate the *PD* or I_{sc} of bladders bathed by HCO_3^--rich solutions in M and S.

The concentration of amiloride required to increase HCO_3^- reabsorption to half-maximal levels (i.e., $3 \cdot 10^{-5}$ M) was between 10 and 100 times more than that required to decrease the Na reabsorption by 50% in the turtle bladder [17], toad bladder [11], and mammalian kidney [16]. At similar concentrations (ca. 10^{-4} M) in rat adipocyte preparations, amiloride has been found to decrease glucose transport and interfere with certain hormonally-induced decreases in carbohydrate, protein, and fat metabolism [14, 15]. Whereas these data show that higher concentrations of amiloride inhibit more than Na^+ transport, the present evidence on HCO_3^- reabsorption is unique insofar as it reflects a stimulatory rather than an inhibitory effect of higher levels of amiloride.

The conclusions from these data on the turtle bladder are the following: (i) Amiloride stimulates the active reabsorption of HCO_3^- ions and this effect is independent of the inhibition of Na^+ transport by this drug. Consequently, amiloride is not a modifier of cation transport [11, 16] alone, as has been conventionally assumed, (ii) To account for all of the observations, one is forced to invoke some interaction(s) between amiloride and the apical membrane other than an increase in the positive surface charge density. Although such an increase in surface charge could account for the direction of change in *PD*, the stimulation of HCO_3^- transport (Table II), and the inhibition of Na^+ transport [17], it cannot account for the lack of effect on the M-to-S flux of Cl^-, whether this Cl^- flux is driven by an electrogenic pump [3, 8, 18] or an electroneutral anion exchanger [9, 10].

The similarity of the amiloride and norepinephrine-induced [1, 2] sti-

mulations of HCO_3^--transport in the turtle bladder would suggest that their actions involve common mechanisms in the apical membrane [2].

Acknowledgement

Amiloride and its analogue was kindly supplied by Dr. E.J. Cragoe of Merck Sharp and Dohme, West Point, Pa. This study was supported by grants from the NIH (1 R01 AM-16928-03) and NSF (PCM76-02344). Dr. G. Ehrenspeck was supported by U.S.P.H.S. Training grant (1 T32-EY07014 -02).

The authors wish to show their appreciation to Mrs. Cristina Matons and Mrs. S. Ehrenspeck for their expert technical assistance.

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