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Effect of amiloride on cell volume regulation in renal straight proximal tubules

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Amiloride has been shown to impair cell volume regulatory decrease in amphiuma red cells. The present study has been performed to test for the influence of amiloride on volume regulatory decrease and electrical properties in isolated perfused mouse straight proximal tubules. Replacement of 40 mmol/l NaCl with 80 mmol/l mannitol in bath perfusate does not appreciably affect the cell volume or the potential difference across the basolateral cell membrane. Reduction of osmolarity by omission of mannitol leads to cell swelling by $16.7 \pm 0.7\%$ ($n = 7$), followed by volume regulatory decrease to $107.2 \pm 1.2\%$ ($n = 7$) of original cell volume within 2 min. 1 mmol/l amiloride (but not 0.1 mmol/l amiloride) in the bath depolarizes the basolateral cell membrane from -63 ± 1 mV ($n = 24$) by $+16 \pm 1$ mV ($n = 16$), decreases the apparent potassium transference number from 0.69 ± 0.02 ($n = 5$) to 0.36 ± 0.05 ($n = 5$), and significantly impairs volume regulatory decrease without appreciably modifying cell volume in isotonic solutions. 1 mmol/l amiloride in the luminal perfusate leads to a slight hyperpolarization of the basolateral cell membrane but does not interfere with volume regulatory decrease. Reduction of bath osmolarity depolarizes the basolateral cell membrane within 30 s by $+7.8 \pm 0.8$ mV ($n = 18$) in the absence and by $+18 \pm 2$ mV ($n = 8$) in the presence of amiloride. In the presence of reduced bath osmolarity and amiloride the potassium transference number amounts to 0.36 ± 0.04 ($n = 8$). The hyperpolarization following luminal application of amiloride is most likely due to inhibition of luminal sodium channels, whereas bath amiloride depolarizes the basolateral cell membrane by reduction of basolateral potassium selectivity. As in amphiuma red cells amiloride impairs volume regulatory decrease in proximal straight renal tubules.

Introduction

Previous studies in our laboratory have shown that exposure of proximal straight tubules of mouse kidney to hypotonic bath perfusates increases the bicarbonate selectivity and decreases the potassium selectivity of the basolateral cell membrane [1], while chloride conductance was minimal in tubules exposed to either hypotonic or isotonic bath perfusates. Furthermore, cell volume regulatory decrease in perfused tubule segments is dependent on the presence of sodium and of bicarbonate but is completely unaffected by 20 min preperfusion with chloride free perfusates on both sides of the epithelium [2]. Thus, volume regulatory decrease in proximal straight tubules apparently involves some bicarbonate-dependent process.

The only cells reported so far involving bicarbonate in volume regulatory decrease are amphiuma erythrocytes: In those cells volume reg-

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ulatory decrease may be accomplished by parallel operation of K^+/H^+ exchange and Cl^-/HCO_3^- exchange [3]. Volume regulatory decrease in those cells can be impaired by amiloride [3].

The present study has been designed to test for an influence of amiloride on volume regulatory decrease in straight proximal tubules. The results indeed show that volume regulatory decrease can be blocked by amiloride.

Methods

The experiments were performed on proximal straight tubules of Swiss mice weighing 20–25 g. Segments of 0.2 to 0.4 mm length were dissected and perfused following principally the method of Burg et al. [4]. Modifications of the technique concerning track system, pipette arrangement, use of a dual channel perfusion pipette, and the electrical circuits for the registration of the potential difference across the basolateral cell membrane (PD_{bl}) have been described in previous publications [5,6]. The luminal perfusion rate was greater than 10 nl/min. The bath was perfused continuously at a rate of 20 ml/min and thermostated with a dual channel feedback system (W. Hampel, Frankfurt, F.R.G.) at a temperature of 38°C. The composition of the perfusates is given in Table I. The bath perfusates were constantly gassed with a mixture of 95% O_2 and 5% CO_2. Amiloride was added to the solutions in the corresponding experiments at a concentration of 1 mmol/l and 0.1 mmol/l, respectively.

Before, during and after exposure to hypotonic solutions, photographs were taken at a magnification of 400× using DIC (Nomarski) contrast (ICM 405 microscope, Zeiss, Oberkochen, F.R.G.) focussed on the center of the tubule. The negatives were enlarged 50-fold and mean outer radius (r) and cell height (h) were determined in each print of one cycle. The apparent cell volume per unit tubule length (V) was calculated from:

$$V = \pi[r^2 - (r-h)^2] = \pi(2rh - h^2)$$

The obtained values are expressed in fractions of the apparent volume (V_0) calculated for tubules prior to hypotonic swelling. The potential difference across the basolateral cell membrane was measured by a high impedance electrometer (FD 223, WPI, Science Trading, Frankfurt, F.R.G.) connected with the electrode via an Ag/AgCl half cell. The electrodes used for recording the potential difference across the basolateral cell membrane were pulled from filament capillaries (1.5 mm o.d., 1.0 mm i.d., Hilgenberg, Malsfeld, F.R.G.) with a Narishige PE 2 vertical puller which was adjusted to deliver electrodes with a resistance between 100 and 200 MΩ. They were filled with 1 mol/l KCl solution immediately before use. For penetrating the membrane the electrodes were advanced rapidly by a piezoelectric stepper (M. Frankenberger, Germering, F.R.G.) mounted on a Leitz micromanipulator (E. Leitz, Wetzlar, F.R.G.). A recording was accepted only, when the penetration of the cell membrane resulted in an instantaneous deflection of the reading. Furthermore, the potential difference across the cell membrane had to be more negative than −50 mV and stable (±2 mV) for at least 1 min. Withdrawal of the electrode was to be followed by an immediate return of the electrode reading to the baseline value (±2 mV). The resistance of the electrodes was checked by short current pulses and had to be constant during the impalement (±20%). The apparent transference number for potassium (tk), i.e. the apparent contribution of peritubular potassium conductance to the conductance of both cell membranes [6] has been calculated from:

$$tk = -dPD \lg(C_1/C_2)/61.5$$

where dPD is the rapid change of the potential difference across the basolateral cell membrane upon increase of bath potassium concentration from 5 (C_1) to 20 mmol/l (C_2).

The data are expressed as arithmetic means ± S.E. Statistical analysis was made by paired t-test, where applicable, $P < 0.05$ was considered statistically significant.

Results

During control conditions (solutions 1 and 6, Table I) the potential difference across the basolateral cell membrane (PD_{bl}) is −63.4 ± 1.3 mV ($n = 24$). No change of PD_{bl} is observed when 40

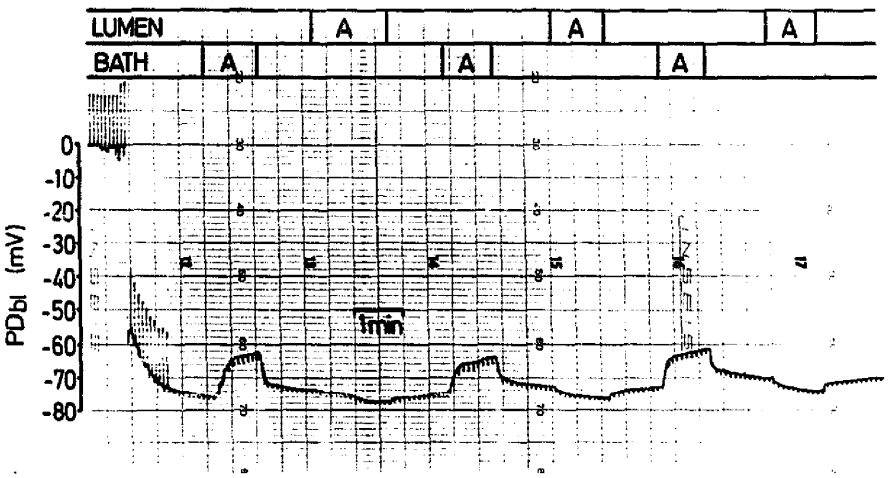

Fig. 1. Effect of 1 mmol/l amiloride added to either the luminal or bath perfusate on the potential difference across the basolateral cell membrane (PD_{bl}) of isolated perfused straight proximal tubules (original tracing). The depolarizing voltage deflections at the beginning of the recording correspond to the input resistance of the electrode, the hyperpolarizing voltage deflections are due to current injections into the tubular lumen.

mmol/l NaCl in solution 1 are replaced by 80 mmol/l mannitol (solution 2, Table I). As shown in Figs. 1 and 2, 1 mmol/l amiloride in the bath perfusate depolarizes the basolateral cell membrane by $+15.9 \pm 1.3$ mV ($n = 16$). 0.1 mmol/l amiloride in the bath does not significantly alter PD_{bl} ($+0.3 \pm 0.3$ mV, $n = 5$), 1 mmol/l amiloride in luminal perfusate hyperpolarizes the basolateral cell membrane by -3.3 ± 0.3 mV ($n = 9$).

As illustrated in Figs. 3 and 4, an increase of bath potassium concentration from 5 to 20 mmol/l (solution 4, Table I) depolarizes the basolateral cell membrane by $+25.5 \pm 0.9$ mV ($n = 5$) in the absence and by $+13.2 \pm 1.9$ mV ($n = 5$) in the presence of 1 mmol/l amiloride in the bath. The respective transference numbers for potassium (tk) amount to 0.69 ± 0.02 and 0.36 ± 0.05, respectively. Decrease of peritubular osmolarity by 80 mosmol/l by omission of mannitol (solution 3, Table I) depolarizes the basolateral cell membrane within 30 s by $+7.8 \pm 0.8$ mV ($n = 18$) in the absence and by $+17.9 \pm 1.7$ mV ($n = 8$) in the presence of 1 mmol/l amiloride in the bath perfusate. In the presence of reduced bath osmolarity

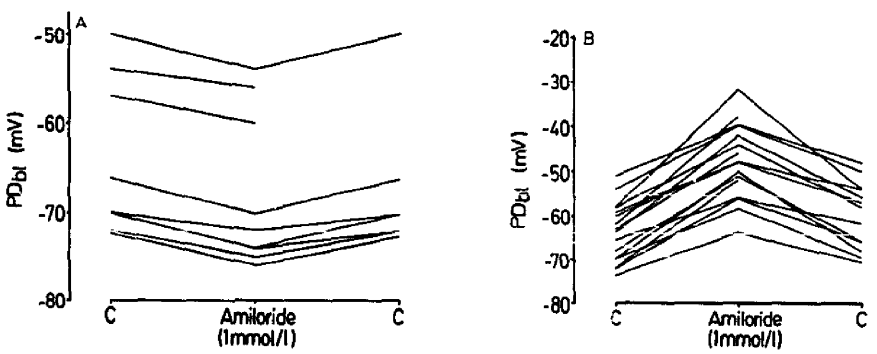

Fig. 2. Effect of 1 mmol/l amiloride added to either the luminl (A) or bath (B) perfusate on the potential difference across the basolateral cell membrane (PD_{bl}) of isolated perfused straight proximal tubules. Each line represents one tubule.

TABLE I

COMPARISON OF SOLUTIONS USED

All solutions were equilibrated with 5% CO_2 and 95% O_2.

Solution	Composition (mmol/l)					
	Bath					Lumen
	1	2	3	4	5	6
NaCl	110	70	70	55	55	120
KCl	5	5	5	20	20	5
$NaHCO_3$	20	20	20	20	20	20
$CaCl_2$	1.3	1.3	1.3	1.3	1.3	1.3
$MgCl_2$	1	1	1	1	1	1
Na_2HPO_4	2	2	2	2	2	2
Sodium acetate	10	10	10	10	10	–
Glucose	5	5	5	5	5	–
Mannitol	–	80	–	80	–	5
Mosmol/l	308	308	228	308	228	308

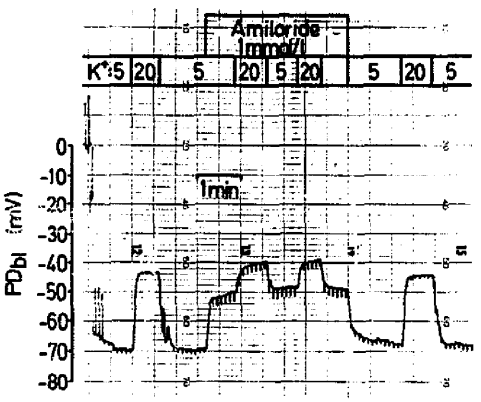

Fig. 3. Effect of bath potassium concentration altered from 5 to 20 mmol/l on the potential difference across the basolateral cell membrane (PD_{bl}) of isolated perfused straight proximal tubules in the presence and in the absence of 1 mmol/l amiloride (original tracing). The depolarizing voltage deflections at the beginning of the recording correspond to the input resistance of the electrode, the hyperpolarizing voltage deflections are due to current injections into the tubular lumen.

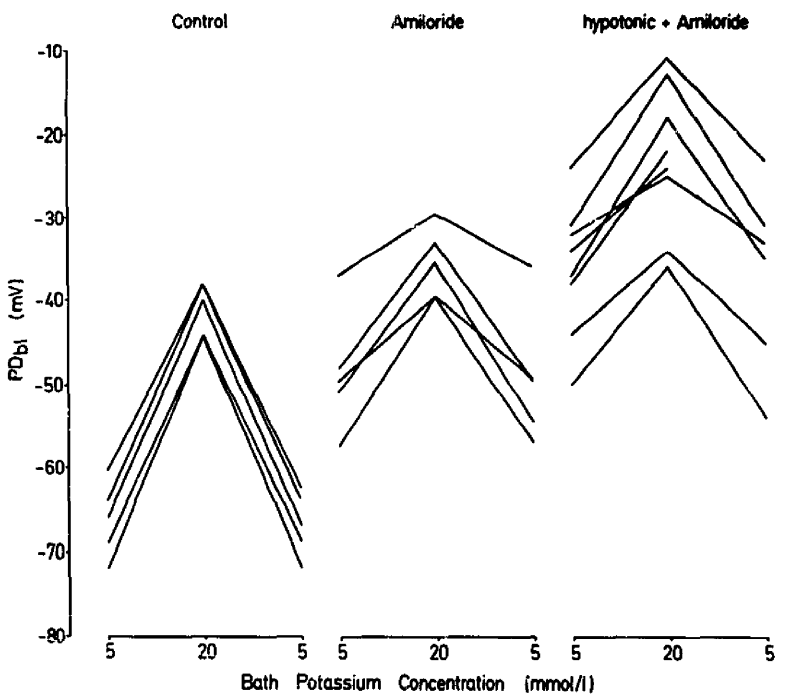

Fig. 4. Effect of bath potassium concentration altered from 5 to 20 mmol/l on the potential difference across the basolateral cell membrane (PD_{bl}) of isolated perfused straight proximal tubules in the absence (left panel) and in the presence of 1 mmol/l amiloride at isotonic conditions (middle panel) and hypotonic conditions (right panel). Each line represents one tubule.

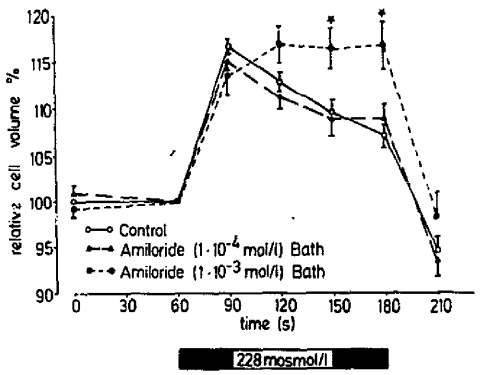

Fig. 5. Effect of hypotonic solutions on cell volume in isolated perfused proximal tubule segments in the absence of amiloride (open circles, $n = 7$), in the presence of 1 mmol/l amiloride (closed circles, $n = 10$) or of 0.1 mmol/l amiloride (closed triangles, $n = 11$) in the bath (mean values ± S.E.).

and amiloride, increase of extracellular potassium concentration from 5 to 20 mmol/l depolarizes the cell membrane by $+13.4 \pm 1.5$ mV ($n = 8$). Accordingly, the apparent transference number for potassium (tk) amounts to 0.36 ± 0.04 ($n = 8$).

Decrease of peritubular osmolarity by 80 mosmol/l by omission of mannitol (solution 3, Table I) leads to a swelling of the tubule epithelium by $16.7 \pm 0.7\%$ ($n = 7$) followed by cell volume regulatory decrease to $107.2 \pm 1.2\%$ of original cell volume within 2 min (Fig. 5). Amiloride (1 mmol/l) does not significantly alter

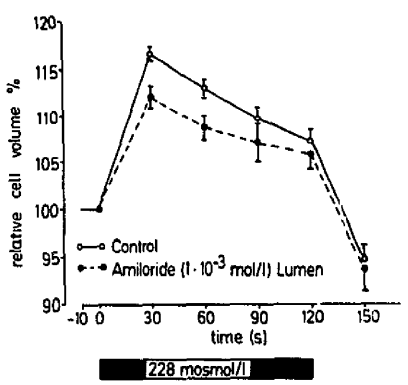

Fig. 6. Effect of hypotonic solutions on cell volume in isolated perfused proximal tubule segments in the absence of amiloride (open circles, $n = 7$) and in the presence of 1 mmol/l amiloride (closed circles, $n = 7$) in the lumen (mean values ± S.E.).

cell volume ($100.9 \pm 0.8\%$, $n = 10$) but severely impairs volume regulatory decrease: In the presence of 1 mmol/l amiloride, cell volume is still $116.8 \pm 2.6\%$ ($n = 10$) of original cell volume 2 min following exposure to hypotonic bath perfusates (Fig. 5). Volume regulatory decrease is not affected significantly by 0.1 mmol/l amiloride in the bath (Fig. 5), or amiloride in the lumen (Fig. 6).

Discussion

The present observations show that amiloride from the luminal cell side hyperpolarizes the basolateral cell membrane and that amiloride from the basolateral cell membrane depolarizes the basolateral cell membrane and markedly impairs regulatory volume decrease.

The slight hyperpolarization of the basolateral cell membrane following application of amiloride to the luminal cell membrane is best explained by inhibition of sodium channels in the luminal cell membrane [7,8,9], leading to reduced circular current across the basolateral cell membrane [10].

The depolarization of the basolateral cell membrane following addition of amiloride to the bath is readily explained by the reduction of potassium selectivity, reflected by the decrease of the apparent transference number for potassium. A decrease of potassium selectivity could result from a decrease of basolateral potassium conductance or an increase of some other conductive pathway.

Amiloride is known to inhibit the sodium/hydrogen ion exchanger [7,11]. A sodium/hydrogen ion exchanger has been reported to occur in the basolateral cell membrane of amphibian proximal tubules [12]. As long as such a system operates in basolateral cell membranes of mammalian proximal tubules and its inhibition leads to intracellular acidification, a decrease of potassium conductance could be explained by intracellular acidification, since potassium conductance in proximal tubules has been shown to depend on intracellular pH [13,14]. A similar effect of amiloride has been observed in amphibian distal tubule [15]. Alternatively, amiloride could reduce potassium conductance by a more direct interference with the potassium channels, although such an effect has not been described so far. This

would explain the rapid, reversible depolarization of the cell membrane following application of amiloride. Other effects ascribed to amiloride do probably not explain the observed depolarization: Inhibition of Na^+/K^+-ATPase [16,17] by amiloride would, similar to ouabain [6], lead to a slow, gradual decline of cell membrane potential to much lower values, with slow recovery. Inhibition of α- and β-receptors [18] is not relevant since no hormones were present in the bath. Amiloride sensitive voltage gated calcium channels [19] are not likely to be present in proximal renal tubules at resting cell membrane potential and increase of intracellular calcium activity, e.g. by inhibition of the sodium/calcium exchanger, would not affect the potassium channels in mouse proximal tubules, since the channels appear to be rather insensitive to alterations of intracellular calcium activity [20].

The mechanism accounting for inhibition of volume regulatory decrease by amiloride is again not entirely clear: One possible mechanism is the inhibition of potassium conductance by amiloride, as suggested in a most recent study on pheochromocytome cultured cells [21]: In a previous study we have shown that barium, which blocks potassium conductance completely and depolarizes the cell membrane to some −30 mV [20], similarly impairs volume regulatory decrease [2]. Furthermore, cells swell following increase of bath potassium concentration and are unable to volume regulate at 30 or 40 mmol/l bath potassium concentration. However, if the cell membrane is depolarized to −40 mV by enhancement of bath potassium concentration to 20 mmol/l, volume regulatory decrease is virtually unaffected (unpublished observations). Amiloride reduces potassium conductance only to half and depolarizes the cell membrane only to slightly less than −50 mV. Thus, inhibition of potassium conductance and the respective depolarization of the basolateral cell membrane may not suffice to explain the marked impairment of volume regulatory decrease.

In conclusion, amiloride from the lumen hyperpolarizes the basolateral cell membrane in mouse straight proximal renal tubules probably by inhibiting sodium channels in the luminal cell membrane. Amiloride from the basolateral side depolarizes the cell membrane by reducing the basolateral cell membrane potassium conductance. Amiloride impairs volume regulatory decrease by reducing the potassium conductance or by interference with some other volume regulatory mechanism.

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References

1 Völkl, H. and Lang, F. (1988) Pflügers Arch. 411, 514–519.
2 Völkl, H. and Lang, F. (1988) Pflügers Arch. 412, 1–6.
3 Cala, P.M. (1983) J. Gen. Physiol. 82, 761–784.
4 Burg, M., Grantham, J., Abramov, M. and Orloff, J. (1966) Am. J. Physiol. 210, 1293–1298.
5 Greger, R. and Hampel, W. (1981) Pflügers Arch. 389, 175–176.
6 Völkl, H., Geibel, J., Greger, R. and Lang, F. (1986) Pflügers Arch. 407, 252–257.
7 Benos, D.J. (1982) Am. J. Physiol. 242, C131–C145.
8 Gögelein, H. and Greger, R. (1986) Pflügers Arch. 406, 198–203.
9 Horisberger, J.-D. and Giebisch, G. (1987) Renal Physiol. 10, 198–220.
10 Lang, F., Messner, G. and Rehwald, W. (1986) Am. J. Physiol. 250, F953–F962.
11 Aronson, P.S. (1985) Annu. Rev. Physiol. 47, 545–560.
12 Boron, W.F. and Boulpaep, E.L. (1983) J. Gen. Physiol. 81, 29–52.
13 Bello-Reuss, E. (1982) J. Physiol. 326, 49–63.
14 Biagi, B., Sohtell, M. and Giebisch, G. (1981) Am. J. Physiol. 241, F677–F686.
15 Oberleithner, H., Münich, G., Schwab, A. and Dietl, P. (1986) Am. J. Physiol. 251, F66–F73.
16 Renner, E.L., Lake, J.R., Cragoe, E.J.Jr. and Scharschmidt, B.F. (1988) Biochim. Biophys. Acta 938, 386–394.
17 Soltoff, S.P. and Mandel, L.J. (1983) Science 220, 957–959.
18 Howard, M.J., Mullen, M.D. and Insel, P.A. (1987) Am. J. Physiol. 253, F21–F25.
19 Tang, C.-M., Presser, F. and Morad, M. (1988) Science 240, 213–215.
20 Völkl, H., Greger, R. and Lang, F. (1987) Biochim. Biophys. Acta 900, 275–281.
21 Delpire, E., Duchene, C., Cornet, M. and Gilles, R. (1988) Pflügers Arch. 411, 223–225.