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Amiloride inhibits proximal tubular reabsorption in conscious euvolemic rats

Klaus Thomsen [a,*], Thomas E.N. Jonassen [b], Sten Christensen [b], David G. Shirley [c]

[a] *Institute for Basic Psychiatric Research, Department of Biological Psychiatry, Aarhus University Hospital, Skovagervej 2, DK-8240 Risskov, Aarhus, Denmark*

[b] *Department of Pharmacology, University of Copenhagen, Copenhagen, Denmark*

[c] *Centre for Nephrology, Royal Free and University College Medical School, London, UK*

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Abstract

Based on the results of micropuncture studies, it is generally assumed that amiloride inhibits Na^+ (and Li^+) reabsorption in the distal nephron, without affecting proximal tubular reabsorption. This is the basis for the use of amiloride to test for distal nephron Li^+ reabsorption. We have examined the validity of this assumption by administering amiloride in doses of 0, 0.02, 0.07, 0.2 and 2.0 mg kg^{-1} h^{-1} to conscious, chronically instrumented rats fed a diet with a normal Na^+ and K^+ content. Na^+ and water homeostasis was maintained by servo-controlled replacement in order to avoid any effect of volume depletion on proximal tubular reabsorption. The effects of the two highest doses of amiloride were also examined without Na^+ and water replacement. In the servo-controlled rats, the two highest doses of amiloride increased the fractional excretion of both Na^+ (FE_{Na}) and Li^+ (FE_{Li}), whereas the two lowest doses affected only FE_{Na}. In the rats without servo-control, FE_{Li} also rose in response to amiloride infusion, but the increase was significantly lower than that observed in the servo-controlled animals. Since distal Li^+ reabsorption is absent or negligible in rats fed a diet with a normal Na^+ and K^+ content, the large increase in FE_{Li} following the highest doses of amiloride (15–18% of the filtered load in servo-controlled rats) indicates inhibition of proximal tubular reabsorption. We conclude that amiloride, in doses usually employed to detect distal Li^+ reabsorption, inhibits proximal tubular reabsorption in conscious euvolemic rats.

Keywords: Amiloride; Li^+ clearance; NHE3; Proximal tubular reabsorption; Na^+ excretion fractional

1. Introduction

Under normal circumstances, filtered Li^+ ions are reabsorbed in the proximal tubule but not in the distal nephron. In consequence, Li^+ clearance (C_{Li}) is widely used as an index of proximal tubular fluid output (V_{prox}) (Thomsen and Shirley, 1997). Under certain conditions, however, some Li^+ reabsorption *does* occur in the distal nephron, and this can be unmasked by the administration of amiloride (Thomsen and Leyssac, 1986; Kirchner, 1987, 1989; Fransen et al., 1992; Shalmi and Thomsen, 1993; Thomsen et al., 1993; Walter et al., 1995; Shirley and Walter, 1997; Thomsen and Shalmi, 1997; Shalmi et al., 1998). Although high concentrations of amiloride can inhibit proximal tubular Na^+/H^+ exchange in vitro (Counillon et al., 1993; Wu et al., 1996), it is generally assumed that the effect of low-dose amiloride, as used in vivo, is confined to the late distal tubules and collecting ducts, where it blocks the luminal Na^+ channels (Benos, 1982; Horisberger and Giebisch, 1987). The latter action is responsible for the inhibition of distal Li^+ reabsorption.

The assumption that in vivo administration of amiloride affects only the distal nephron is supported by micropuncture studies. During Na^+ and/or K^+ depletion, where distal Li^+ reabsorption has been documented, amiloride at a dose of 2 mg kg^{-1} h^{-1} increases C_{Li} without affecting directly measured V_{prox} (Kirchner, 1987, 1989; Fransen et al., 1992; Walter et al., 1995; Shirley and Walter, 1997), whereas the same dose of amiloride has no effect on C_{Li}, or on directly measured V_{prox}, in animals fed on a normal diet, where no distal Li^+ reabsorption occurs (Shirley et al., 1992; Walter et al., 1992; Leyssac and Christensen, 1994). In contrast, however, we and others have observed in a number of studies in *conscious* rats on a normal diet that

* Corresponding author. Tel.: +45-7789-3512; fax: +45-7789-3549.
E-mail address: klausth@post6.tele.dk (K. Thomsen).

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this dose of amiloride increases the fractional excretion of Li^+ (FE_{Li}) by 1–7% of the filtered load (Petersen and DiBona, 1992; Shalmi and Thomsen, 1993; Thomsen et al., 1993; Thomsen and Shalmi, 1997; Shalmi et al., 1998; Grønbeck et al., 1998). These observations raise the possibility that amiloride can affect reabsorption in the proximal tubule, but that the effect can be seen only in conscious rats because they are less likely to be in a Na^+-retaining state than are rats prepared for micropuncture.

The aim of the present study was to determine whether in vivo administration of amiloride does affect Na^+ reabsorption in the proximal tubules. If the effect depends on the degree to which Na^+-retaining mechanisms are activated, it may be seen more clearly during servo control of Na^+ and water balance, where fluid depletion is avoided. We have therefore examined the effect of a range of doses of amiloride on FE_{Li} in conscious, unstressed rats maintained on a normal diet, in the presence and absence of servo-control of their Na^+ and water balance.

2. Materials and methods

2.1. Animals and physical environment

Specific pathogen-free female Wistar rats (200–260 g) were obtained from M&B, Ry, Denmark. The studies were carried out in accordance with the European Community guidelines and approved by the Danish Animal Experiments inspectorate. The animals were housed in a temperature- (22–24 °C) and moisture- (60%) controlled room with a 12 h light–dark cycle. The rats were fed a wet mash diet containing 200 mmol of Na^+ and 100–200 mmol of K^+ per kg dry weight for at least 10 days prior to experimentation. For 3 days prior to the clearance study, Li^+ citrate was added to the food (12 mmol/kg dry weight) to obtain measurable plasma [Li^+] without influencing renal function (Shalmi and Thomsen, 1989).

2.2. Surgical preparation

Ten days before experimentation, the animals were anaesthetized with Hypnorm® (Janssen Pharmaceutica, Belgium) (Fentanyl citrate 0.315 mg/ml + Fluanisone 10 mg/ml, 400 μl/kg) + Dormicum® (Hoffmann-La Roche, Switzerland) (Midazolam 5 mg/ml, 800 μl/kg). Using aseptic surgical techniques, sterile Tygon catheters were advanced into the abdominal aorta and the inferior vena cava via the femoral vessels, and a sterile chronic suprapubic bladder catheter was implanted into the bladder. All catheters were produced and fixed, with small modifications, as described by Petersen et al. (1991). After instrumentation, the rats were given 0.9% NaCl solution s.c. (5 ml) and a long-lasting analgesic (Temgesic; Reckitt and Coleman, Hull, UK, 10 μg/animal, s.c.). The arterial and venous catheters were sealed with 50% glucose solution containing 500 units of heparin and 10,000 units of streptokinase per milliliter. After regaining consciousness, the rats were returned to the animal unit and housed individually. After a recovery period of 5–6 days, the rats were acclimatised to restraining cages in order to avoid stress during the investigation (Petersen et al., 1991). The training consisted of three daily sessions with a stepwise increase in duration from 1 to 3 h.

2.3. Clearance protocol

2.3.1. Series 1

The rats were examined according to the following scheme: each experiment comprised a 15-min bolus period and a 105-min equilibration period, for infusion of markers, followed by three 20-min urine collection periods for estimating baseline (pre-amiloride) values. This was followed by infusion of amiloride during 60 min of equilibration and during three 20-min urine collection periods for estimating the effect of amiloride.

The experiments were carried out between 9 a.m. and 2 p.m., with the conscious rats immobilised in restraining cages. The rats were connected to a Baxter Uniflow blood pressure transducer via the arterial catheter. Through the pressure transducer, a continuous intra-arterial infusion of 25 mM glucose solution containing heparin (100 units/ml) at a rate of 5 μl/min was given to keep the arterial catheter open. Through the venous catheter, the animals received an infusion of 25 mM glucose solution throughout the experiment (bolus 0.6 ml, sustained 10 μl/min) containing [^{14}C] tetraethylammonium bromide (TEA) (New England Nuclear, Boston, USA) (bolus 0.84 μCi, sustained 0.014 μCi/min), [^{3}H]-inulin (Amersham International, Aylesbury, UK) (bolus 1.8 μCi, sustained 0.03 μCi/min) and LiCl (bolus 7.2 μmol, sustained 120 nmol/min). Amiloride (Merck) was dissolved in demineralised water and given at a rate of 10 μl/min in doses of 0 (time controls), 0.02, 0.07 0.2 or 2 mg kg^{-1} h^{-1}. In addition, 25 mM glucose was given intravenously throughout the experiment at a rate adjusted so that the total infusion rate of the above-mentioned solutions, including that given in the artery, was maintained at a rate of 45 μl/min in order to keep an adequate minimum urine flow rate necessary for elimination of bladder emptying errors.

During the final 120 min of the experiment, fluid and Na^+ balance was maintained by a computer-driven servo-control system (Spannow et al., 1997). From the bladder catheter, urine passed a Na^+-sensitive electrode, which performed one measurement of urinary [Na^+] per second (Nova-biochemical, Waltham, MA, USA). Urine was collected in vials arranged in an auto-sampler placed on an electronic balance. The auto-sampler was operated by a photocell, which allowed change of the vial without touching the balance. Data on urine production (weight on scale) and urinary [Na^+] were sampled continuously on an IBM-compatible computer, which in turn controlled the infusion rates of two independent infusion pumps that delivered 25 mM glucose solution and 300 mM NaCl solution, respectively.

Water balance was calculated taking into account the 45 μl/min of glucose solution given continuously throughout the study and the variable amount of fluid given by the NaCl solution pump. The servo control system for water balance was adjusted to infuse 15 μl/min less than the excretion rate in order to avoid any volume expansion that might otherwise have arisen during periods when the urine flow rate was less that the basal infusion rate of 45 μl/min. Pilot studies have shown that without this precaution, there is a tendency to a self-sustaining increase in urine output. Urinary output of Na^+ and fluid were integrated over 5 min, thus allowing a 5-min delay in changes of Na^+ and glucose infusion rates.

Blood samples of 250 μl were collected from the arterial catheter at the start and end of each 1 h (3×20 min) urine collection period. All blood samples were replaced immediately with heparinized donor blood.

2.3.2. Series 2

These rats were treated in the same way as those in series 1, but no servo control of Na^+ and water balance was instituted during amiloride infusion. Only two doses of amiloride (0.2 and 2.0 mg kg^{-1} h^{-1}) were used.

2.4. Na^+ electrode

The Na^+ electrode was calibrated with standard solutions containing 10 and 100 mM NaCl solution in 5 mM KCl solution. Since the signal was linearly related to ln [Na^+], no further NaCl standards were needed. It was maintained in good condition and its stability monitored as described previously (Thomsen et al., 1999). After the experiment, the Na^+ excretion measured by the servo system was compared with the Na^+ excretion based on conventional measurements of urinary Na^+ excretion (using flame emission photometry) and the weight of the urine samples collected. In every case, there was excellent agreement between measurements obtained by the two methods.

2.5. Analyses

Urine volume was determined gravimetrically. [Na^+] and [K^+] in plasma and urine and [Li^+] in plasma were determined by flame emission photometry. [Li^+] in urine was determined by atomic absorption photometry. [^{14}C]-TEA and [^{3}H]-inulin activities in plasma and urine were determined by liquid scintillation counting on a Packard Tri-Carb liquid scintillation analyzer, mixing 15 μl of the sample and 285 μl of water with 2.5 ml of Ultima Gold (Packard Instruments, Meriden, CT, USA).

2.6. Calculations

Renal clearances (C) and fractional excretions (FE) were calculated by the standard formulas:

$$C = U * V/P; FE = C/\mathrm{GFR},$$

where U is the urine concentration, V is the urine flow rate, P is the plasma concentration and GFR is the glomerular filtration rate as measured by ^{3}H-inulin clearance.

2.7. Data presentation and statistics

All values are presented as mean ± S.E.M. The values for renal clearance variables are derived from the averages of the

Table 1
Renal clearance data before and during amiloride infusion in rats *with* servo control of Na^+ and H_2O balance (mean ± S.E.M.)

Amiloride dose (mg kg^{-1} h^{-1})		0	0.02	0.07	0.2	2.0
Number of rats		9	9	6	6	6
Body weight (g)		264 ± 8	238 ± 4	232 ± 4	236 ± 4	239 ± 5
C_{TEA} (μl min^{-1} 100 g^{-1} bw)	pre-amiloride	–	3070 ± 178	2635 ± 38	2600 ± 73	2901 ± 234
	amiloride	–	3029 ± 233	2720 ± 138	2871 ± 52	3045 ± 233
C_{In} (μl min^{-1} 100 g^{-1} bw)	pre-amiloride	837 ± 39	832 ± 42	768 ± 24	826 ± 38	868 ± 68
	amiloride	802 ± 44	902 ± 54	777 ± 42	863 ± 37	853 ± 51
C_{In}/C_{TEA} (%)	pre-amiloride	–	26.8 ± 1.4	28.9 ± 0.8	30.8 ± 1.8	30.6 ± 1.8
	amiloride	–	29.0 ± 0.9[a]	28.6 ± 1.0	28.3 ± 1.1	28.7 ± 1.6
FE_{Li} (%)	pre-amiloride	31.7 ± 2.8	38.0 ± 1.3	29.0 ± 2.0	33.9 ± 2.7	28.2 ± 2.1
	amiloride	34.5 ± 2.7	38.6 ± 2.6	32.6 ± 2.5[a]	49.3 ± 3.6[c]	45.9 ± 2.3[c]
FE_{Na} (%)	pre-amiloride	0.28 ± 0.09	0.60 ± 0.06	0.25 ± 0.03	0.57 ± 0.12	0.38 ± 0.11
	amiloride	0.24 ± 0.06	1.03 ± 0.16[a]	0.83 ± 0.19[a]	2.31 ± 0.33[b]	2.07 ± 0.46[a]
C_{Na}/C_{Li} (%)	pre-amiloride	0.8 ± 0.2	1.6 ± 0.1	0.9 ± 0.1	1.7 ± 0.3	1.2 ± 0.3
	amiloride	0.6 ± 0.1	2.6 ± 0.3[b]	2.6 ± 0.6[a]	4.7 ± 0.6[b]	4.3 ± 0.7[b]
FE_K (%)	pre-amiloride	11.3 ± 1.1	14.0 ± 1.3	7.9 ± 1.4	9.3 ± 2.3	7.3 ± 1.8
	amiloride	12.0 ± 1.2	10.4 ± 1.6	1.9 ± 0.7[c]	2.2 ± 0.7[b]	0.8 ± 0.1[a]
FE_V (%)	pre-amiloride	2.3 ± 0.1	2.8 ± 0.2	2.7 ± 0.1	3.2 ± 0.2	2.7 ± 0.2
	amiloride	2.6 ± 0.1	3.6 ± 0.7	3.2 ± 0.2	4.2 ± 0.6	4.0 ± 0.6

bw, body weight; C_{TEA}, tetraethylammonium clearance; C_{In}, inulin clearance; FE_{Li}, fractional Li^+ excretion; FE_{Na}, fractional Na^+ excretion; C_{Na}/C_{Li}, fractional distal Na^+ excretion; FE_K, fractional K^+ excretion; FE_V, fractional water excretion. Pre-amiloride versus amiloride (Student's paired t-test): [a]$P < 0.05$; [b]$P < 0.01$; [c]$P < 0.001$.

three pre-amiloride periods and the three periods during amiloride infusion, respectively. Overall statistical comparisons were performed by two-way analysis of variance for repeated measures for two-way classified data (dose and time). Individual comparisons within each dose level were performed by subsequent use of Student's paired *t*-test and individual comparisons between doses or series were performed by unpaired *t*-test. Differences were considered statistically significant at the 0.05 level.

3. Results

3.1. Rats with servo-control of Na^+ and H_2O balance (series 1)

Mean arterial blood pressure and plasma electrolyte concentrations were similar before and during treatment with amiloride (data not shown).

Renal clearance data are given in Table 1. Amiloride did not affect the effective renal plasma flow (C_{TEA}), glomerular filtration rate (C_{In}) or effective filtration fraction (C_{In}/C_{TEA}). In contrast, the fractional excretion of Li^+ (FE_{Li}) showed an increase in response to amiloride. The effect was dose-dependent and reached statistical significance at a dose of 0.07 mg kg^{-1} h^{-1} (although at this dose, the increase was no different from that seen in time controls). The change in FE_{Li} seen at the highest dose amounted to 18% of the filtered load. The fractional excretion of Na^+ (FE_{Na}) also showed a dose-dependent increase in response to amiloride; the change reached statistical significance at a dose of 0.02 mg kg^{-1} h^{-1}. C_{Na}/C_{Li} also rose significantly at a dose of 0.02 mg kg^{-1} h^{-1} and reached a maximum level at a dose of 0.2 mg kg^{-1} h^{-1}. The fractional excretion of K^+ (FE_K) decreased markedly in response to amiloride. The apparent decrease with the lowest dose did not reach statistical significance, but a dose of 0.07 mg kg^{-1} h^{-1} produced almost a maximal effect. Although there was a tendency for the fractional excretion of water to increase following amiloride treatment, the increase was not statistically significant at any individual dose.

Fig. 1 compares the effects of amiloride on FE_{Li}, FE_{Na}, and FE_K. It appears that, compared to the group given no amiloride, a dose of 0.07 mg kg^{-1} h^{-1} did not affect FE_{Li}, whereas it led to significant changes in FE_{Na} and FE_K. In contrast, further increases of the amiloride dose, to 0.2 and 2 mg kg^{-1} h^{-1}, led to pronounced increases of both FE_{Na} and FE_{Li}, but no further decrease in FE_K.

3.2. Rats without servo-control of Na^+ and H_2O balance (series 2)

Series 2 rats were examined in the same way as those of series 1 except that the servo-control for Na^+ and water was not switched on and only the two highest doses of amiloride were examined. This series was included in order to assess the effect of volume status on the response to amiloride. At the highest dose, there was a negative water balance of 2073 ± 568 μl and a negative Na^+ balance of 372 ± 59 μmol at the end of the 2 h of amiloride administration. Both FE_{Li} and FE_{Na} rose significantly (Table 2), but the increase in FE_{Li} was significantly lower ($P<0.05$) than the corresponding increase seen in series 1. At the lower dose (0.2 mg kg^{-1} h^{-1}), there were slightly smaller negative water and Na^+ balances (1514 ± 711 μl and 300 ± 53 μmol, respectively) after amiloride administration. Again the increase in FE_{Li} was lower than the correspond-

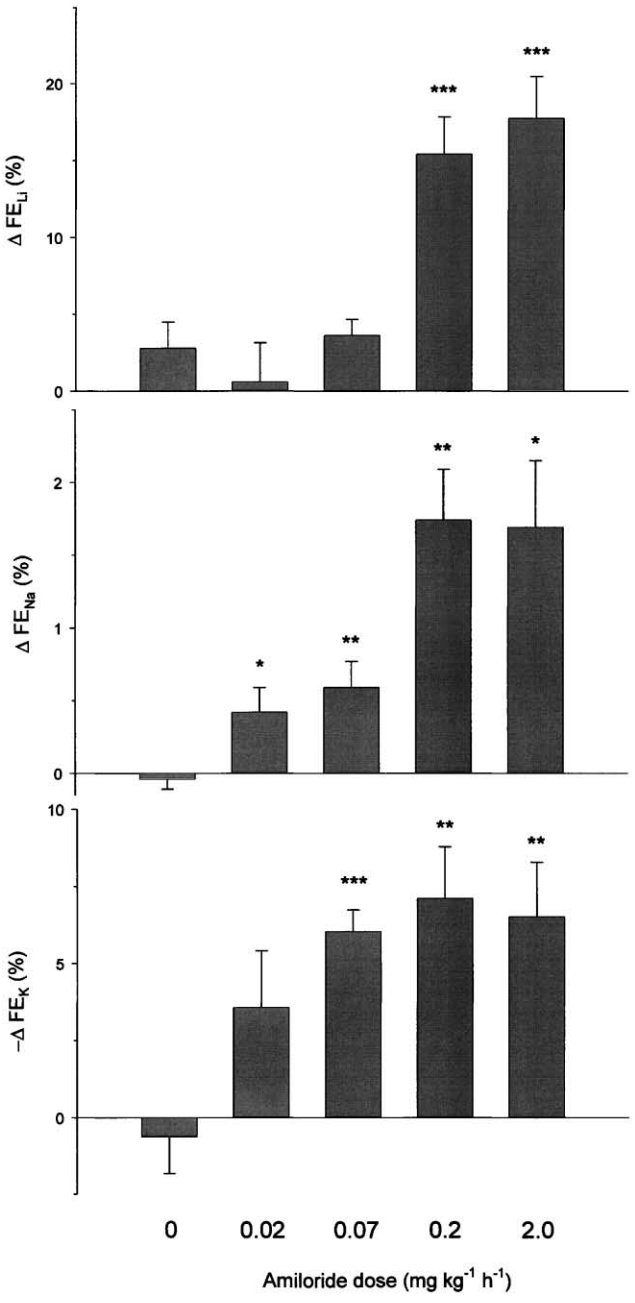

Fig. 1. Change in fractional excretion of Li^+, Na^+ and K^+ (%) in response to amiloride in rats with servo-controlled Na^+ and fluid balance. Amiloride versus no amiloride: * $P<0.05$; ** $P<0.01$; *** $P<0.001$ (Student's *t*-test).

Table 2
Renal clearance data before and during amiloride infusion in rats *without* servo control of Na^+ and H_2O balance (mean ± S.E.M.)

Amiloride dose (mg kg^{-1} h^{-1})		0.2	2.0
Number of rats		4	5
Body weight (g)		242 ± 3	240 ± 4
C_{TEA} (μl min^{-1} 100 g^{-1} bw)	pre-amiloride	2591 ± 277	2066 ± 102
	amiloride	3018 ± 75	2585 ± 113[a]
C_{In} (μl min^{-1} 100 g^{-1} bw)	pre-amiloride	771 ± 72	718 ± 40
	amiloride	739 ± 106	763 ± 58
C_{In}/C_{TEA} (%)	pre-amiloride	33.8 ± 0.4	35.8 ± 2.6
	amiloride	30.8 ± 0.5	32.6 ± 1.4
FE_{Li} (%)	pre-amiloride	25.4 ± 2.4	28.0 ± 2.7
	amiloride	33.2 ± 4.1	37.7 ± 2.0[b]
FE_{Na} (%)	pre-amiloride	0.22 ± 0.04	0.27 ± 0.10
	amiloride	1.01 ± 0.16[b]	1.37 ± 0.22[c]
C_{Na}/C_{Li} (%)	pre-amiloride	0.85 ± 0.10	0.86 ± 0.25
	amiloride	3.00 ± 0.17[c]	3.61 ± 0.46[c]
FE_K (%)	pre-amiloride	7.0 ± 0.6	9.0 ± 1.0
	amiloride	0.6 ± 0.2[b]	0.5 ± 0.1[c]
FE_V (%)	pre-amiloride	3.0 ± 0.3	3.1 ± 0.4
	amiloride	3.5 ± 0.5	3.5 ± 0.3

bw, body weight; C_{TEA}, tetraethylammonium clearance; C_{In}, inulin clearance; FE_{Li}, fractional Li^+ excretion; FE_{Na}, fractional Na^+ excretion; C_{Na}/C_{Li}, fractional distal Na^+ excretion; FE_K, fractional K^+ excretion; FE_V, fractional water excretion. Pre-amiloride versus amiloride (Student's paired *t*-test): [a]$P<0.05$; [b]$P<0.01$; [c]$P<0.001$.

ing increase seen in series 1, although in this case, the difference did not achieve statistical significance.

4. Discussion

The main finding of the present study in conscious, Na^+-replete rats was that FE_{Li} rose in response to infusion of amiloride and the effect was dose-related. In principle, the increase in FE_{Li} could be due to an effect on the distal nephron or to an effect on the proximal tubule, or to a combination of the two. However, micropuncture studies have shown that Li^+ is not reabsorbed to any significant extent in the amiloride-sensitive distal nephron segment unless the dietary Na^+ or K^+ intake is extremely low. In the present study, the dietary Na^+ and K^+ contents were high, well above the values that lead to significant distal nephron reabsorption of Li^+ (Thomsen and Shalmi, 1997); and the increases of FE_{Li} observed during administration of the two highest doses of amiloride (up to 18% of the filtered load) were far beyond the maximum increase (<6%) that could arise from inhibition of any putative distal nephron Li^+ reabsorption occurring in rats on a normal Na^+ and K^+ diet (Thomsen and Shirley, 1997). Such high increases could only be derived from increased proximal tubular fluid output.

This conclusion is supported by the observation in series 1 that with the two lowest doses of amiloride, there was an effect on FE_{Na} but not on FE_{Li}, whereas with the two higher doses, there were effects on both FE_{Li} and FE_{Na}. Furthermore, FE_K appeared to be suppressed almost to a maximal degree at a dose of 0.07 mg kg^{-1} h^{-1}, whereas this dose was far from being maximal with regard to effects on FE_{Li} and FE_{Na}. This suggests that the effect of amiloride on the distal nephron was maximal at a dose of 0.07 mg kg^{-1} h^{-1}, whereas the additional effects of amiloride on FE_{Na} and FE_{Li} observed at higher doses were due to an effect on the proximal tubule.

In a previous study with similar urine flow rates and a dose of amiloride equal to the highest dose in the present study (2 mg kg^{-1} h^{-1}), we measured an average urinary amiloride concentration of 170 ± 18 μmol/l and excretion rate of 65 ± 8 nmol min^{-1} kg^{-1} (Shalmi et al., 1998). From these data, intratubular amiloride concentrations were estimated to be 5–10 μmol/l in the proximal tubules and at least five times higher in the cortical collecting ducts. These values are at the lower end of the range of concentrations that inhibit the Na^+/H^+ exchange mechanism (NHE) in the apical membrane of the proximal tubule (in vitro K_i = 30–100 μmol/l for NHE3) (Counillon et al., 1993; Wu et al., 1996) but well above the concentrations required to block the apical Na^+ channels in the distal nephron (in vitro K_i < 1 μmol/l) (Benos, 1982; Horisberger and Giebisch, 1987). On this basis, we would expect complete inhibition of Na^+ reabsorption through principal cells in the distal nephron, but only a moderate effect on reabsorption in the proximal tubule — as was indeed observed in the present study. The reason why this dose of amiloride did not show any inhibitory effect on proximal tubular Na^+ reabsorption in micropuncture studies (Shirley et al., 1992; Walter et al., 1992; Leyssac and Christensen, 1994) may be related to the fact that Na^+-retaining mechanisms are activated by acute anaesthesia and surgery (Thomsen and Olesen, 1981). In contrast, our rats were euvolemic, conscious and unstressed owing to the preceding training (Petersen et al., 1991).

The somewhat lower increase in FE_{Li} in response to amiloride in series 2 compared with that in series 1 supports the idea that the increase in FE_{Li} is dampened by amiloride-induced loss of Na^+ and suggests that the full effect of amiloride on the proximal tubule can only be unmasked by servo control of Na^+ and water balance. The increase in FE_{Li} in series 2 was slightly above the increase of up to 7% of the filtered load found previously (Petersen and DiBona, 1992; Shalmi and Thomsen, 1993; Thomsen et al., 1993; Thomsen and Shalmi, 1997; Shalmi et al., 1998; Grønbeck et al., 1998). This may be due to a higher volume status in the present series resulting from the rather high infusion rate of fluid of 45 μl/min versus 20 μl/min used in previous studies.

As indicated in the Introduction, the use of FE_{Li} as an index of fractional proximal tubular fluid excretion is based on the condition that no Li^+ is reabsorbed in the distal nephron. Unfortunately, this condition is not always met and in some circumstances Li^+ is also reabsorbed to some extent by the amiloride-sensitive Na^+ channel in the late distal tubules and cortical collecting ducts. Because it blocks this channel, amiloride is used as a tool to examine whether or not in a given situation Li^+ is reabsorbed in the distal

nephron. Based on micropuncture results, it has been common practice to interpret any amiloride-induced increase in FE_{Li} as an indication of distal nephron Li^+ reabsorption. However, the present results show that, at least in conscious rats, this interpretation must be modified because amiloride, in doses normally used to block distal nephron Li^+ reabsorption, also inhibits proximal tubular reabsorption, which itself leads to an increase of FE_{Li}.

In conclusion, this study has provided firm evidence that administration of amiloride in vivo can inhibit reabsorption in the proximal tubules as well as the in the distal nephron. With respect to Li^+ clearance studies, our results do not imply that amiloride must be abandoned as a tool to reveal distal nephron Li^+ reabsorption. However, they do indicate that the presence of distal nephron Li^+ reabsorption in a given experimental situation can only be established if the change in FE_{Li} in response to amiloride is higher than that observed in an appropriate control group.

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