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Evidence for a magnesium-sparing action by amiloride during renal clearance studies in rats

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1 The potassium-sparing diuretic, amiloride, reduced the fractional excretion of magnesium in anaesthetized rats.

2 Alterations in glomerular filtration rate (GFR), the filtered load of magnesium, arterial blood pressure, the status of the extracellular fluid volume, plasma aldosterone concentration and acid-base balance were not involved.

3 It was concluded that amiloride exerted a magnesium-sparing effect by a direct renal action.

Introduction

In addition to the excretion of sodium and water, diuretic agents may also alter the renal handling of other electrolytes. One of the most common and serious side-effects of diuretic therapy is an increased urinary loss of potassium, particularly associated with the more potent diuretic agents. In attempts to prevent this, potassium-sparing diuretics such as amiloride may be administered concomitantly with the more potent diuretics. Another, although less well publicized, side-effect of diuretic therapy is the excessive loss of urinary magnesium also particularly associated with the potent loop-blocking diuretics (Duarte, 1968a; Lim & Jacob, 1972; Ryan & Phillips, 1977). Although the effects of potassium-sparing diuretics on renal magnesium handling have not been widely studied, a number of earlier studies suggested that these drugs might reduce urinary magnesium excretion (Horton & Biglieri, 1962; Hanze & Seyberth, 1967; Counihan, Dunne, Halley, Ryan & Ryan, 1978; Lim & Jacob, 1978).

In a previous study we have shown that amiloride reduced the urinary excretion of magnesium ($U_{Mg}V$) in conscious saline-loaded rats (Devane & Ryan, 1981). However under the experimental conditions employed in that study, it was not possible to establish whether the reduced urinary magnesium excretion was due to a direct action of amiloride on renal function or whether it was secondary to changes in such factors as the extracellular fluid volume (ECFV), the filtered load of magnesium, haemodynamic parameters or hormonal influences. In an effort to determine whether such indirect factors were involved, we have now extended our investigations to the effects of amiloride on the renal clearance of magnesium in anaesthetized rats. In this model the status of the extracellular fluid volume may be controlled and monitored, glomerular filtration rate (GFR) and filtered loads measured and corrected for, mean arterial blood pressure recorded and plasma aldosterone levels measured. Some of our findings have been presented in preliminary form (Devane & Ryan, 1980).

Methods

Clearance experiments were performed in male Wistar rats, weighing about 300 g, anaesthetized by intraperitoneal injection of inactin (sodium-5-ethyl-5-(1-methyl-propyl)-2-thiobarbiturate; 100 mg per kg body weight). On induction of anaesthesia, the left femoral artery was catheterized and an arterial blood sample (120 μl) was taken for an initial estimate of arterial haematocrit. This initial value in inactin-anaesthetized rats has been shown to be a reliable index of the plasma haematocrit of conscious rats (Ichikawa, Maddox, Cogan & Brenner, 1978). The arterial catheter was subsequently used for sampling arterial blood and monitoring arterial blood pressure.

An infusion of donor rat plasma was begun into the cannulated right jugular vein (2.4 ml h^{-1} for the first hour; thereafter 0.4 ml h^{-1}). A similar protocol has been shown to maintain plasma volume during preparatory surgery (Ichikawa *et al.*, 1978). A bolus injection of 6 μCi [^{3}H]-inulin in 0.6 ml of 0.9% w/v NaCl solution was given into the left jugular vein, followed by an infusion of a modified Krebs-Ringer

solution (composition mM: Na 145.0, K 4.5, Mg 0.8, Ca 1.5, Cl 124.5, HCO_3 25.0, PO_4 1.5 and SO_4 1.0) containing 1.2 μCi ml^{-1} [^{3}H]-inulin, at a rate of 30 ml kg^{-1}h^{-1}. This solution was aerated with 95% O_2 and 5% CO_2, pH 7.4.

The trachea was cannulated. The left ureter was catheterized with polyethylene tubing (PP 10) and urine was collected between oil, into calibrated constant bore capillary tubes. After 90–100 min equilibration, a series of five consecutive 30 min urine collections were made. Arterial blood samples (120 μl) were taken at the beginning and end of each urine collection. Femoral arterial blood pressure was also monitored at the beginning and end of each urine collection by means of a strain gauge pressure transducer (Bioscience PT 400) and recorded on an ink writing oscillograph. After the final urine collection, the animals were bled by puncture of the abdominal aorta.

Plasma ultrafiltrates were obtained by filtration of 1 ml of plasma through Amicon membranes (Centriflo, 50,000 mol. wt$_r$). Samples of plasma were also stored frozen for the subsequent measurement of aldosterone levels.

Time-control experiments

Clearance studies were performed in seven control animals, in order to establish the baseline pattern of kidney function and electrolyte excretion during the period of investigation.

Amiloride experiments

The effects of amiloride on renal electrolyte clearance were studied in five animals. After two 30 min control urine collections were made, amiloride (2.0 mg kg^{-1}h^{-1}) was added to the modified Ringer infusion solution. Three 30 min experimental urine collections were then made. An infusion of amiloride was chosen in preference to a bolus injection as rapid injections of amiloride have been reported to lower blood pressure and to produce anuria in anaesthetized dogs (Duarte, 1968b). Additional blood samples (100 μl) were taken, once during the control period and once during the experimental period, for blood pH measurement.

Analytical methods

Plasma and urine were analysed for sodium and potassium concentrations by flame emission spectrophotometry. Plasma and plasma ultrafiltrate and urine was analysed for magnesium concentrations by atomic absorption spectrophotometry, using the Pye Unicam SP 90A spectrophotometer in each case. Plasma and urine [^{3}H]-inulin concentrations were measured by liquid scintillation counting (Intertechnique SL 20). Blood pH was measured with a capillary glass electrode at 37°C using an Ultra-micro pH and gas analyser (Instrumentation Lab. Inc. 113-01). Plasma aldosterone concentrations were measured by radioimmunoassay using a Cis Aldosterone RIA kit with an ^{125}I tracer (Aldoctk-125) and a Beckman 4000 gamma counter.

Calculations

Results refer only to the left kidney and calculations were made as follows:

glomerular filtration rate (GFR) = $V(\frac{U}{P}\text{In})$;

fractional excretion (FE) = $(\frac{U}{P_{Uf}})$ / $(\frac{U}{P}\text{In})$; where

V = urine flow rate (ml min^{-1}),

$\frac{U}{P}$In = ratio of urine to plasma [^{3}H]-inulin concentration and

$\frac{U}{P_{Uf}}$ = ratio of urine to ultrafilterable plasma electrolyte concentration.

All numerical data are expressed as means ± s.e.mean. For the purpose of statistical analysis, a control observation (mean of two 30 min periods) and an experimental observation (mean of three 30 min periods) were calculated for each animal. The control and experimental data within the time-control and amiloride-treated groups were analysed statistically using the paired Student's *t* test.

Results

The results of studies in time-control animals are summarised in Table 1. There were no statistically significant differences between control and experimental periods for GFR, urinary volume and the fractional excretion of sodium ($\%FE_{Na}$) and magnesium ($\%FE_{Mg}$). The fractional excretion of potassium ($\%FE_K$) did increase slightly during the experimental period ($P < 0.05$). Mean arterial blood pressure and plasma potassium concentration were constant from control to experimental periods, but plasma magnesium concentration did fall slightly ($P < 0.05$) as did the haematocrit values ($P < 0.01$). The initial blood haematocrit value (taken immediately after induction of anaesthesia) was 42.1 ± 1.3% (mean ± s.e.mean).

An infusion of amiloride (see Table 2) did not change GFR, but urine volume and $\%FE_{Na}$ increased significantly ($P < 0.05$ and $P < 0.01$; respectively). Both $\%FE_K$ ($P < 0.001$) and $\%FE_{Mg}$ ($P < 0.01$) fell during amiloride infusion whilst mean arterial blood pressure, plasma magnesium concentration, blood pH and haematocrit values remained unchanged.

Table 1 Measurements in time-control rats of glomerular filtration rate (GFR), urinary volume, fractional excretion (%FE) of electrolytes, plasma electrolyte concentrations, blood pressure and haematocrit

	Control	*Experimental*	P *value*
GFR ($ml\,min^{-1}$)	0.98 ± 0.07	0.96 ± 0.06	NS
Urine volume ($\mu l\,min^{-1}$)	4.8 ± 1.0	5.2 ± 0.7	NS
$\%FE_{Na}$	0.87 ± 0.26	0.97 ± 0.22	NS
$\%FE_{K}$	36.9 ± 2.6	42.6 ± 2.5	<0.05
$\%FE_{Mg}$	14.0 ± 1.4	14.9 ± 0.9	NS
Plasma K (mM)	4.14 ± 0.10	3.97 ± 0.09	NS
Plasma Mg (mM)	0.87 ± 0.03	0.82 ± 0.02	<0.05
Mean arterial pressure (mmHg)	128 ± 2	127 ± 3	NS
Blood haematocrit	43.0 ± 0.8	39.6 ± 0.9	<0.01

All results are expressed as the mean ± s.e.mean. Experiments were carried out in 7 rats, anaesthetised with inactin, whose mean body weight was 297 ± 23 g. Two control periods (2×30 min) were followed by three experimental periods (3×30 min) during each of which data were collected. Statistical significance was assessed using a paired Student's *t* test; NS, $P > 0.05$.

Table 2 The effects of amiloride ($2.0\,mg\,kg^{-1}h^{-1}$) on glomerular filtration rate (GFR), urine volume, fractional excretion (%FE) of electrolytes, plasma electrolyte concentrations, blood pressure and haematocrit

	Control	*Amiloride*	P *value*
GFR ($ml\,min^{-1}$)	0.99 ± 0.11	0.96 ± 0.09	NS
Urine Volume ($\mu l\,min^{-1}$)	5.5 ± 0.9	9.0 ± 1.3	<0.05
$\%FE_{Na}$	0.57 ± 0.13	2.28 ± 0.17	<0.001
$\%FE_{K}$	21.1 ± 1.9	3.0 ± 0.7	<0.001
$\%FE_{Mg}$	18.9 ± 4.6	15.9 ± 3.5	<0.01
Plasma K (mM)	4.71 ± 0.23	5.35 ± 0.17	<0.001
Plasma Mg (mM)	0.85 ± 0.01	0.87 ± 0.02	NS
Mean arterial pressure (mm Hg)	121 ± 3	123 ± 2	NS
% Blood haematocrit	41.0 ± 0.5	40.0 ± 0.7	NS
Blood pH*	7.418 ± 0.029	7.425 ± 0.028	NS

All results are expressed as the mean ± s.e.mean. Experiments were carried out in 5 rats, anaesthetised with inactin, whose mean body weight was 315 ± 8 g. After the control periods (2×30 min), amiloride ($2.0\,mg\,kg^{-1}h^{-1}$) was infused during the experimental period (3×30 min). Statistical significance was assessed using a paired Student's *t* test; NS, $P > 0.05$.
* Measured in 3 rats only.

Plasma potassium concentration increased significantly during amiloride infusion ($P < 0.001$). The initial blood haematocrit value in this group of rats was $41.9 \pm 0.8\%$ (mean ± s.e.mean).

Ultrafilterable plasma magnesium and plasma aldosterone concentration values for the time-control and amiloride-treated groups are summarised in Table 3, between which no statistically significant differences occurred. Plasma levels of aldosterone, measured in inactin-anaesthetized rats without saline or diuretic infusions, had an average value of 0.65 nM.

Discussion

The results of the present study confirm our previous observations in conscious saline-loaded rats that amiloride reduced the urinary excretion of magnesium (Devane & Ryan, 1981). Previously, no firm conclusion could be made regarding the mode of action of amiloride in conserving magnesium. The present renal clearance studies were undertaken to assess whether any indirect factors were involved or whether a direct renal action of amiloride caused the reduced renal excretion of magnesium.

Amiloride could have caused an enhanced reabsorption of magnesium from the proximal tubule in response to a reduction of the extracellular fluid volume, secondary to its diuretic and natriuretic effects. In the present clearance studies, however, the fluid and electrolyte infusion protocol was adequate to prevent such a contraction of the extracellular fluid volume. Indeed, the blood haematocrits measured during the 'experimental' period were lower than the initial values of the time-control animals and the haematocrits remained unchanged during infusion of amiloride. In addition, the rapid time course of the magnesium conserving effect of amiloride and the constancy of GFR and mean arterial blood pressure during its infusion argue against a mechanism involving a reduction of extracellular fluid volume.

Alternatively it was possible that amiloride caused a reduced renal excretion of magnesium secondary to a fall in the filtered load of magnesium. In the present study, GFR and plasma magnesium concentration remained unchanged during infusion of amiloride. The percentage of ultrafilterable plasma magnesium did not differ significantly between time-control and amiloride-treated animals and the values for the two groups were in good agreement with those published (Brunette & Crochet, 1975; Sachtjen, Meyer & Massry, 1979). Thus, the observed fall in urinary excretion of magnesium could not be attributed to amiloride causing a fall in the filtered load of magnesium.

Administration of amiloride in congestive heart failure patients has been shown to raise plasma levels of aldosterone (Counihan, Dunne, Ryan & Ryan, 1979). The elevated aldosterone levels were observed to follow a rise in plasma potassium concentration due to the urinary potassium retaining effect of amiloride. Thus it was considered possible that the observed urinary magnesium-sparing effect of amiloride could be secondary to an aldosterone-mediated increase in the reabsorption of sodium and fluid. Although in the present study, infusion of amiloride did result in an increase in plasma potassium concentration, there was no difference in plasma aldosterone levels between the time-control group and the amiloride treated group. In addition the rapid time-course of the magnesium-sparing action argues also against the involvement of aldosterone in this effect. The elevated plasma aldosterone levels observed in both groups of animals were probably related to induction of anaesthesia, and the stress of surgery as has been previously found (Pettiger, Tanaka, Keeton, Campbell & Brooks, 1975; Cochrane, 1978; Oyama, Toniguchi, Jin, Satone & Kudo, 1979).

Table 3 Comparison of ultrafilterable plasma magnesium and plasma aldosterone concentrations in time-control and amiloride-treated animals

	Time-control	*Amiloride*	P *value*
% Ultrafilterable Mg	81.0 ±2.3	80.6 ±2.6	NS
Plasma aldosterone (nM)	2.61 ± 0.29	2.81 ± 0.33	NS
n	7	5	

The results are expressed as the mean ± s.e.mean. Ultrafilterable plasma magnesium and plasma aldosterone concentrations were measured in each animal from blood taken at the end of the investigation period. Statistical significance was assessed using an unpaired Student's *t* test. NS, $P > 0.05$.

Mean arterial blood pressure did not change on infusion of amiloride and similarly GFR remained constant. Although alterations in other haemodynamic factors could have occurred following infusion of amiloride it was considered unlikely that alterations in renal haemodynamics were involved in the urinary magnesium conserving effect of amiloride. The possibility that amiloride might have caused a reduced urinary excretion of magnesium through alterations in acid/base balance was considered unlikely since blood pH did not appear to change following amiloride administration.

Since amiloride did not appear to induce any of the indirect effects which have been considered as possible mechanisms of a reduced urinary excretion of magnesium, it was concluded that a direct renal action of amiloride was probably involved. Such an action could conceivably have come about by either an enhanced reabsorption of magnesium, or a reduced secretion of magnesium.

Most micropuncture studies indicate that the distal tubule plays a minor role in the renal handling of magnesium, accounting for the reabsorption of at most only 5–10% of the filtered load, with no evidence for net secretion of magnesium (Brunette, Vigneault & Carriere, 1974; Quamme, Wong, Dirks, Roinel, De Rouffignac & Morel, 1978). However, earlier clearance studies and some more recent micropuncture studies suggest that net secretion of magnesium can occur in the distal tubule (Averill & Heaton, 1966; Wenn, Wong & Dirks, 1971; Rios, Ingram, Ingram & Di Bona, 1977; Lechene & Blouch 1979).

The generally accepted view of the diuretic action of amiloride suggests a primary action to reduce the permeability of the luminal membrane of the distal tubule epithelium to sodium. The small flux of sodium then causes a fall in the transepithelial potential difference and a reduction in the electrochemical gradient favouring potassium secretion. Such a reduction in lumen negativity would also be expected either to favour the reabsorption of other cations such as magnesium or to reduce magnesium secretion.

The magnitude of the urinary magnesium conserving effect observed in this present clearance study was considerably smaller than that observed previously in conscious saline-loaded rats, where an approximately 50% reduction in urinary magnesium excretion ($U_{Mg}V$) occurred (Devane & Ryan, 1981). The reason for this is not clear. The intravenous infusion of amiloride used in the present study was as effective in terms of the magnitude of its natriuretic and urinary potassium retaining effects, as the oral administration of 2–5 mg kg^{-1} used previously. It is possible that the conditions of the present clearance study which involved a relatively vigorous fluid infusion protocol might have resulted in a reduced contact time and blunted an amiloride-induced increase in the reabsorption of magnesium. Alternatively, since the reabsorption of magnesium in the distal tubule has been shown to be load-dependent (Quamme & Dirks, 1980), it is possible that the delivery of magnesium to the distal tubule was a limiting factor in the present studies.

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