Br. J. Pharmac. (1983), **80**, 421–428

Dose-dependent reduction in renal magnesium clearance by amiloride during frusemide-induced diuresis in rats

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1 The effects of the potassium-sparing diuretic, amiloride, on fractional magnesium excretion were investigated at four doses (0.02, 0.08, 0.20 and 2.00 mg $kg^{-1} h^{-1}$) during frusemide-induced diuresis.

2 Amiloride caused a dose-dependent reduction in the fractional excretion of both magnesium and potassium, whereas the effects of amiloride, over the same dose range, on fractional sodium and calcium excretion were not dose-dependent.

3 The results indicate that amiloride exerted a specific renal action to alter magnesium and potassium transport dissociated from effects on sodium excretion.

Introduction

Amiloride, a potassium-sparing diuretic, is believed to act in the distal tubule of the nephron, where it is reported to exert a mild diuretic and natriuretic action and a marked potassium-sparing effect (Baba, Lont, Smith, Townshend & Wilson, 1968; Duarte, Chomety & Giebisch, 1971).

We have previously reported that amiloride reduced the urinary excretion of magnesium (UV_{Mg}) in conscious saline-loaded rats (Devane & Ryan, 1981a). We have also confirmed these results in anaesthetized rats in which amiloride reduced the fractional excretion of magnesium (FE_{Mg}), and have provided evidence that amiloride exerted its magnesium-sparing action by a direct renal action (Devane & Ryan, 1980).

Amiloride is most commonly used in combination with more effective diuretic agents in order to minimize the excessive loss of potassium in the urine associated with conventional potent diuretic therapy. However diuretics such as frusemide also result in large urinary losses of other electrolytes such as magnesium (Duarte, 1968a; Lim & Jacob, 1972; Ryan & Phillips, 1977). In a study in congestive heart failure patients receiving frusemide, amiloride administration has been reported to cause a small but significant reduction in the urinary excretion of magnesium with a corresponding increase in plasma and lymphocyte levels of magnesium (Counihan, Dunne, Halley, Ryan & Ryan, 1978; Ryan, Ryan & Counihan 1981). The present clearance studies therefore were undertaken to assess the magnitude of and test for dose-response characteristics of a urinary magnesium-sparing action by amiloride in anaesthetized rats during a constant frusemide diuresis. Some of our findings have been presented in preliminary form (Devane & Ryan, 1981b).

Methods

Clearance experiments were performed in male Wistar rats. The animals were anaesthetized by intraperitoneal injection of Inactin (sodium-5-ethyl-5-(1)methyl-propyl)-2-thiobarbiturate; 100 mg kg^{-1} 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 arterial haematocrit value in Inactin-anaesthetized rats, has been shown to be a reliable index of the conscious plasma volume (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 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% NaCl was given into the left jugular vein, followed by an infusion of a

modified 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 0.6 μCi ml^{-1} [^{3}H]-inulin, at a rate of 60 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 catherized with polyethylene tubing (PP10), and urine was collected between oil, into calibrated constant bore capillary tubes. After 100 min equilibration, frusemide (1.0 mg kg^{-1} h^{-1}) was added to the modified Ringer infusion solution. After a further 40 min equilibration, a series of six 15-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 using a strain gauge pressure transducer (Bioscience PT400) 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.$_\tau$). Samples of plasma were also stored frozen for the subsequent measurement of aldosterone levels.

Inulin clearance was used to estimate glomerular filtration rate (GFR).

Time-control experiments

Clearance studies were performed in five frusemide-control animals, in order to establish the baseline pattern of kidney function and electrolyte excretion during the period of investigation under the conditions of a frusemide diuresis.

Amiloride experiments

The effects of amiloride on renal electrolyte clearance were investigated at four different doses; 0.02, 0.08, 0.20 and 2.00 mg kg^{-1} h^{-1}. Four groups, with five animals in each group were used. Each group received one dose of amiloride. After three 15-min control-frusemide urine collections were made, amiloride (0.02, 0.08, 0.20 or 2.00 mg kg^{-1} h^{-1}), was added to the modified Ringer infusion solution. Three 15-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 produce anuria in anaesthetized dogs (Duarte, 1968b).

Table 1 Summary of fractional excretion rates (%FE) of sodium, potassium, magnesium and calcium in a time-control group and four-amiloride-treated groups (0.02, 0.08, 0.20 and 2.00 mg kg^{-1} h^{-1}) during a frusemide diuresis (1.0 mg kg^{-1} h^{-1})

Experimental group		*%FE$_{Na}$*	*%FE$_K$*	*%FE$_{Mg}$*	*%FE$_{Ca}$*
Frusemide time-control	C	8.0 ± 0.3	63.1 ± 5.7	40.8 ± 3.2	8.8 ± 1.1
	E	8.1 ± 0.4	68.4 ± 6.0	39.8 ± 2.8	10.4 ± 1.1
		NS	$P < 0.01$	NS	NS
Amiloride (0.02 mg kg^{-1} h^{-1})	C	8.3 ± 0.4	70.0 ± 2.7	35.2 ± 1.6	9.2 ± 0.5
	E	8.9 ± 0.2	51.0 ± 2.4	35.5 ± 1.6	7.9 ± 0.3
		NS	$P < 0.001$	NS	NS
Amiloride (0.08 mg kg^{-1} h^{-1})	C	10.4 ± 0.7	82.3 ± 2.1	43.8 ± 1.4	11.2 ± 1.1
	E	11.1 ± 0.4	43.0 ± 1.4	37.1 ± 0.9	8.1 ± 0.5
		NS	$P < 0.001$	$P < 0.05$	$P < 0.05$
Amiloride (0.20 mg kg^{-1} h^{-1})	C	9.4 ± 0.2	105.9 ± 5.9	48.2 ± 1.6	13.2 ± 0.9
	E	10.9 ± 0.2	34.1 ± 1.8	38.3 ± 1.7	10.3 ± 0.6
		$P < 0.01$	$P < 0.01$	$P < 0.05$	$P < 0.05$
Amiloride (2.00 mg kg^{-1} h^{-1})	C	8.6 ± 0.4	103.6 ± 5.8	42.3 ± 1.6	11.0 ± 0.6
	E	10.6 ± 0.3	23.4 ± 1.2	30.8 ± 1.8	8.7 ± 0.4
		$P < 0.05$	$P < 0.01$	$P < 0.01$	$P < 0.05$

The results are expressed as the mean ± s.e.mean. Paired experiments were carried out in each group of animals. In the time-control group, three control (C) frusemide periods (3 × 15 min) were followed by three experimental (E) frusemide periods (3 × 15 min). In the other groups, after three control (C) frusemide periods (3 × 15 min), amiloride (0.02, 0.08, 0.20 or 2.00 mg kg^{-1} h^{-1} as appropriate)was added to the infusion solution and three experimental (E) urine collections were made. Control and experimental data were compared and statistical significance (P) was assessed using a paired Student's t test. (NS = $P > 0.05$).

Analytical methods

Plasma and urine were analysed for sodium and potassium concentrations by flame emission spectrophotometry. Plasma, plasma ultrafiltrate and urine were analysed for magnesium concentration by atomic absorption spectrophotometry, using a Varian-AA475 atomic absorption spectrophotometer in each case. Plasma and urine [^{3}H]-inulin concentrations were measured by liquid scintillation counting (Intertechnique SL 20). 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.

$$\text{Glomerular filtration rate (GFR)} = V\left(\frac{U}{P}\,\text{In}\right)$$

$$\text{Fractional excretion (FE)} = \left(\frac{U}{P_{Uf}}\right) / \left(\frac{U}{P}\,\text{In}\right)$$

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 the mean ± s.e.mean. For the purposes of statistical analysis, a control observation (mean of three 15-min periods) and an experimental observation (mean of three 15-min periods) was calculated for each animal. The comparison of control and experimental data within the time-control and amiloride-treated groups was analysed statistically using a paired Student's *t* test.

Results

The results of the fractional excretion of sodium, potassium, magnesium and calcium in both the frusemide time-control group and amiloride-treated groups are summarised in Table 1. There were no significant differences between control and experimental periods in the fractional excretion of sodium ($\%FE_{Na}$), magnesium ($\%FE_{Mg}$) and calcium

Table 2 Summary of glomerular filtration rate (GFR), urine volume, mean arterial blood pressure and percentage blood haematocrits in a time-control group and four amiloride-treated groups (0.02, 0.08, 0.20 and 2.00 mg kg^{-1} h^{-1}) during a frusemide diuresis (1.0 mg kg^{-1} h^{-1})

Experimental group		*GFR* (ml min^{-1})	*Urine volume* (μl min^{-1})	*Mean arterial blood pressure* (mmHg)	*% Hct*
Frusemide	C	0.68 ± 0.03	68.3 ± 4.6	129 ± 6	40.9 ± 1.1
time-control	E	0.68 ± 0.03	69.9 ± 4.5	134 ± 4	38.9 ± 0.9
		NS	NS	NS	$P < 0.01$
Amiloride	C	0.64 ± 0.05	65.7 ± 6.0	140 ± 2	41.6 ± 0.3
(0.02 mg kg^{-1} h^{-1})	E	0.66 ± 0.04	66.7 ± 4.0	139 ± 2	40.1 ± 0.3
		NS	NS	NS	$P < 0.01$
Amiloride	C	0.75 ± 0.03	94.2 ± 4.6	146 ± 7	45.3 ± 0.5
(0.08 mg kg^{-1} h^{-1})	E	0.70 ± 0.03	85.8 ± 3.2	149 ± 8	42.7 ± 0.3
		NS	NS	NS	$P < 0.001$
Amiloride	C	0.91 ± 0.02	100.7 ± 3.4	136 ± 2	39.5 ± 0.9
(0.20 mg kg^{-1} h^{-1})	E	0.89 ± 0.03	101.0 ± 3.0	137 ± 2	38.6 ± 0.6
		NS	NS	NS	NS
Amiloride	C	0.78 ± 0.04	84.2 ± 6.7	139 ± 4	40.8 ± 0.4
(2.00 mg kg^{-1} h^{-1})	E	0.73 ± 0.05	85.1 ± 5.2	139 ± 4	39.5 ± 0.4
		NS	NS	NS	NS

The results are expressed as the mean ± s.e.mean. Paired experiments were carried out in each group of animals. In the time-control group three control (C) frusemide periods (3 × 15 min) were followed by three experimental (E) frusemide periods (3 × 15 min). In the other groups, after three control (C) frusemide periods (3 × 15 min), amiloride (0.02, 0.08, 0.20 or 2.00 mg kg^{-1} h^{-1} as appropriate) was added to the infusion solution and three experimental (E) urine collections were made. Control and experimental data were compared and statistical significance (*P*) was assessed using a paired Student's *t* test. (NS = $P > 0.05$).

Table 3 Summary of plasma potassium, magnesium and calcium concentrations in a time-control group and four amiloride-treated groups (0.02, 0.08, 0.20 and 2.00 mg $kg^{-1} h^{-1}$) during a frusemide diuresis (1.0 mg $kg^{-1} h^{-1}$)

Expimental group		*Plasma K* (mmol l^{-1})	*Plasma Mg* (mmol l^{-1})	*Plasma Ca* (mmol l^{-1})
Frusemide	C	3.56±0.08	0.72±0.03	2.15±0.06
time-control	E	3.45±0.09	0.73±0.02	2.21±0.06
		$P<0.05$	NS	$P<0.05$
Amiloride	C	4.13±0.05	0.81±0.01	2.57±0.05
(0.20 mg $kg^{-1} h^{-1}$)	E	4.01±0.03	0.75±0.01	2.61±0.05
		NS	$P<0.01$	NS
Amiloride	C	3.46±0.11	0.79±0.02	2.66±0.05
(0.08 mg $lg^{-1} h^{-1}$)	E	3.79±0.15	0.76±0.01	2.74±0.04
		$P<0.05$	NS	$P<0.01$
Amiloride	C	2.66±0.10	0.62±0.02	2.07±0.08
(0.20 mg $kg^{-1} h^{-1}$)	E	2.91±0.18	0.61±0.02	1.92±0.07
		NS	NS	NS
Amiloride	C	2.73±0.15	0.69±0.02	1.99±0.06
(2.00 mg $kg^{-1} h^{-1}$)	E	3.20±0.18	0.74±0.02	2.17±0.06
		NS	NS	NS

The results are expressed as the mean ± s.e.mean. Paired experiments were carried out in each group of animals. In the time-control group three control (c) frusemide periods (3 × 15 min) were followed by three experimental (E) frusemide periods (3 × 15 min). In the other groups, after three control (C) frusemide periods (3 × 15 min), amiloride (0.02, 0.08, 0.20 or 2.0 mg $kg^{-1} h^{-1}$ as appropriate) was added to the infusion solution and three experimental (E) urine collections were made. Control and experimental data were compared and statistical significance (P) was assessed using a paired Student's t test. (NS = $P>0.05$).

($\%FE_{Ca}$), for the time-control group. The fractional excretion of potassium ($\%FE_K$) did increase slightly during the experimental period ($P<0.05$), in the time control group. A significant increase in $\%FE_{Na}$ occurred only at the two highest doses of amiloride (0.20, $P<0.01$ and 2.00 mg $kg^{-1} h^{-1}$, $P<0.05$). In contrast, infusion of amiloride at all four doses (0.02, 0.08, 0.20 and 2.00 mg $kg^{-1} h^{-1}$) resulted in a significant reduction in $\%FE_K$, ($P<0.01$, $P<0.001$, $P<0.01$ and $P<0.01$ respectively). A significant reduction in $\%FE_{Mg}$ occurred at the three higher doses of amiloride (0.08, $P<0.05$; 0.20, $P<0.05$ and 2.00 mg $kg^{-1} h^{-1}$, $P<0.01$). Similarly a significant reduction in $\%FE_{Ca}$ occurred at the three higher doses of amiloride (0.08, $P<0.05$; 0.20, $P<0.05$ and 2.00 mg $kg^{-1} h^{-1}$, $P<0.05$).

The results of glomerular filtration rate (GFR), urine volume, mean arterial blood pressure and percentage blood haematocrits in the frusemide time-control group and the amiloride treated groups are summarised in Table 2. There was no significant difference between control and experimental periods in GFR, urine volume or mean arterial blood pressure, for the time-control group. Blood haematocrits did fall slightly during the experimental period ($P<0.01$), in the time-control group. Regardless of the dose, infusion of amiloride did not change GFR, urine volume or mean arterial blood pressure. Infusion of amiloride at the two lower doses (0.02 and 0.08 mg $kg^{-1} h^{-1}$) did result in small falls in blood haematocrits ($P<0.01$ and $P<0.001$ respectively). Blood haematocrits remained unchanged during infusion of amiloride at the two higher doses (0.20 and 2.00 mg $kg^{-1} h^{-1}$).

The results of plasma potassium, magnesium and calcium concentrations in the frusemide time-control group and the amiloride-treated groups are summarised in Table 3. In the time-control group there was a minor decrease in plasma potassium concentration ($P<0.05$), a minor increase in plasma calcium concentration ($P<0.05$) and plasma magnesium concentration remained unchanged. Infusion of amiloride at 0.02 mg $kg^{-1} h^{-1}$ resulted in a small decrease in plasma magnesium ($P<0.01$) while plasma potassium and calcium concentrations were unchanged. Infusion of amiloride at 0.08 mg $kg^{-1} h^{-1}$ resulted in small increases in plasma potassium ($P<0.05$) and plasma calcium ($P<0.01$) concentrations while plasma magnesium concentration remained unchanged. Plasma concentrations of potassium, magnesium and calcium remained unchanged during infusion of amiloride at the two higher doses (0.20 and 2.00 mg $kg^{-1} h^{-1}$).

Percentage ultrafilterable plasma magnesium and calcium values and plasma aldosterone concentrations for the frusemide time-control and amiloride-

Table 4 Comparison of percentage ultrafilterable plasma magnesium (% Uf. Mg) and calcium (% Uf. Ca) and also plasma aldosterone concentrations in a time-control group and four amiloride-treated groups (0.20, 0.08, 0.20 and 2.00 mg $kg^{-1} h^{-1}$) during a frusemide diuresis (1.0 mg $kg^{-1} h^{-1}$).

Experimental group	*% Uf. Mg*	*% Uf. Ca.*	*Plasma aldosterone* (nmol l^{-1})
Frusemide time-control	84.3 ± 1.6	69.2 ± 3.6	1.91 ± 0.52
Amiloride (0.20 mg $kg^{-1} h^{-1}$)	84.1 ± 1.4	70.4 ± 4.4	2.41 ± 0.28
Amiloride (0.08 mg $kg^{-1} h^{-1}$)	84.1 ± 1.3	65.6 ± 2.4	2.40 ± 0.28
Amiloride (0.20 mg $kg^{-1} h^{-1}$)	83.0 ± 1.3	58.9 ± 4.5	2.03 ± 0.44
Amiloride (2.00 mg $kg^{-1} h^{-1}$)	86.7 ± 2.1	68.8 ± 3.3	2.50 ± 0.24
P value	NS	NS	NS

The results are expressed as the mean ± s.e.mean. Percentage ultrafilterable plasma magnesium and calcium and also plasma aldosterone concentrations were measured in each animal from blood taken at the end of the investigation period. Statistical significance was assessed using a one-way analysis of variance (NS = $P > 0.05$).

treated groups are summarised in Table 4. Statistical analysis of these data by one-way analysis of variance showed no significant difference for either percentage ultrafilterable plasma magnesium and calcium or plasma aldosterone concentrations.

The magnitude of the effects of amiloride (0.02, 0.08, 0.20 and 2.00 mg $kg^{-1} h^{-1}$) on fractional electrolyte excretion are summarised in Table 5. When assessed by a one-way analysis of variance (ANOVA) and an F test, the magnitude of the effects of amiloride on $\%FE_K$ ($P < 0.01$) and $\%FE_{Mg}$ ($P < 0.01$) showed a significant dose-dependence, whereas the magnitude of the effects of amiloride on $\%FE_{Na}$ and $\%FE_{Ca}$ did not show a significant dose-dependence. The relationship between dose of amiloride and response is shown graphically in Figure 1 for $\%FE_K$ and in Figure 2 for $\%FE_{Mg}$.

Discussion

The results showing that amiloride caused a marked

Table 5 Summary of the magnitude of the effects of amiloride (0.02, 0.08, 0.20 and 2.00 mg $kg^{-1} h^{-1}$) on fractional excretion (Δ %FE) of potassium, magnesium, sodium and calcium during a frusemide diuresis (1.0 mg $kg^{-1} h^{-1}$)

	Amiloride dose (mg $kg^{-1} h^{-1}$)				*Dose-response relationship ANOVA*
	0.02	0.08	0.20	2.00	
n	5	5	5	5	(*P* value)
Δ $\%FE_K$	− 19.0 ± 1.2	− 38.5 ± 3.8	− 72.4 ± 9.8	− 80.3 ± 9.4	< 0.01
P	< 0.001	< 0.001	< 0.01	< 0.01	
Δ $\%FE_{Mg}$	0.2 ± 1.6	− 6.2 ± 1.8	− 9.5 ± 2.8	− 11.5 ± 2.2	< 0.01
P	NS	< 0.05	< 0.05	< 0.01	
Δ $\%FE_{Na}$	0.66 ± 0.78	0.96 ± 0.90	1.48 ± 0.28	1.98 ± 0.77	NS
P	NS	NS	< 0.01	< 0.05	
Δ $\%FE_{Ca}$	1.2 ± 1.0	− 2.7 ± 1.2	− 2.7 ± 0.9	− 2.3 ± 0.7	NS
P	NS	< 0.05	< 0.05	< 0.05	

The results are expressed as the mean ± s.e.mean. Statistical significance at each dose was assessed using a paired Student's *t* test. The statistical significance of the dose-response relationship between amiloride dose and the change in fractional electrolyte excretion (Δ %FE) was assessed using a one-way analysis of variance (ANOVA) and an F test. (NS = $P > 0.05$).

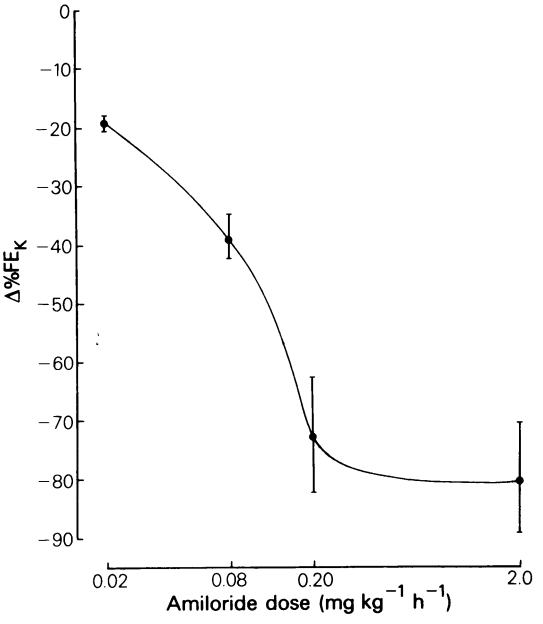

Figure 1 Dose-response relationships between amiloride dose (0.02, 0.08, 0.20 and 2.00 mg kg^{-1} h^{-1}) and the change in fractional excretion of potassium (Δ %FE$_K$). The effects of amiloride were studied during a frusemide-induced diuresis (1.0 mg kg^{-1} h^{-1}). The statistical significance of the dose-response relationship was assessed using a one-way analysis of variance and an F test ($P<0.01$).

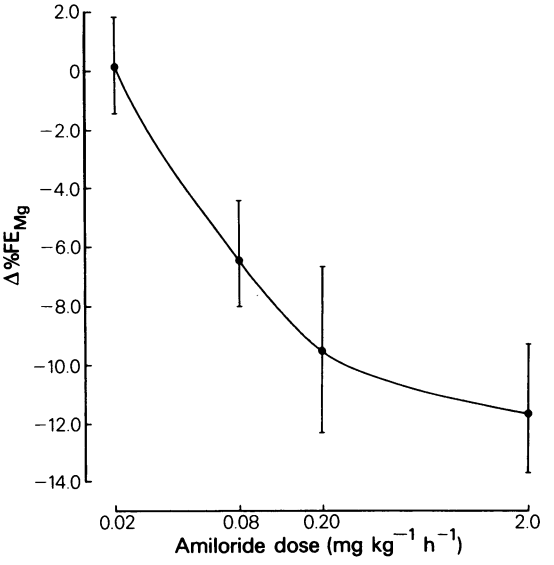

Figure 2 Dose-response relationship between amiloride dose (0.02, 0.08, 0.20 and 2.00 mg kg^{-1} h^{-1}) and the change in fractional excretion of magnesium (Δ %FE$_{Mg}$). The effects of amiloride were studied during a frusemide-induced diuresis (1.0 mg kg^{-1} h^{-1}). The statistical significance of the dose-response relationship was assessed using a one-way analysis of variance and an F test ($P<0.01$).

reduction in urinary potassium excretion during a frusemide-induced diuresis are consistent with out previously reported observations in conscious saline-loaded rats (Devane & Ryan, 1981a). Amiloride at all four doses caused a reduction in the fractional excretion of potassium and the effect showed a significant dose-dependence over the dose range investigated. The effects of amiloride on the fractional excretion of sodium were not as consistent. Only at the two higher doses was an increased fractional excretion of sodium observed.

The generally accepted view of the mechanism of action of amiloride is that the sodium permeability of the luminal membrane of the distal tubular epithelium is reduced. Consequent to this reduced influx of sodium into the tubular epithelium, there is a fall in the transepithelial potential difference and a reduction in the electrochemical gradient favouring potassium secretion. This view of the mechanism of action of amiloride has been inferred primarily from *in vitro* studies in transport systems analogous to the distal tubule such as the frog skin, toad skin and toad bladder (Ehrlich & Crabbe, 1968; Crabbe, 1968; Salako & Smith, 1970; Cuthbert & Shum, 1974). Such a mechanism assumes a primary action by amiloride altering sodium reabsorption. Although only final urine was sampled, the observations in the present studies of a consistent action by amiloride reducing potassium excretion while sodium excretion was not as markedly or as consistently affected, do not support such a theory. A similar dissociation between the effects of amiloride on potassium and sodium excretion has been reported by other workers. In a micropuncture study of the effects of amiloride in the distal tubule, a similar effect of amiloride reducing potassium secretion while causing little or no change in sodium reabsorption was observed (Duarte *et al.*, 1971). These authors suggested that amiloride may have a direct inhibitory effect on the peritubular uptake of potassium available for secretion. Guignard & Peters (1970), found during either a metabolic alkalosis or hypercapnia, that while the urinary potassium-sparing effect of amiloride was still evident, the naturiuretic effect was abolished. These authors suggested that amiloride acted at the luminal membrane of the distal tubule to reduce passive efflux of potassium into the tubular fluid.

Amiloride, at all except the lowest dose, caused a significant reduction in the fractional excretion of magnesium. This finding confirmed our previously reported observations of a magnesium sparing action

by amiloride during frusemide diuresis in saline-loaded rats (Devane *et al.*, 1981a) and also confirmed the clinical observation of a magnesium-sparing action by amiloride in congestive heart failure patients being treated with frusemide (Counihan *et al.*, 1978; Ryan *et al.*, 1981). In a previous clearance study in rats, it was concluded that the effect of amiloride in reducing the fractional excretion of magnesium reflected a direct renal action of amiloride rather than a secondary effect due to contraction of the extracellular fluid volume, a fall in the filtered load of magnesium, a change in mean arterial blood pressure or an alteration in plasma aldosterone levels (Devane *et al.*, 1980). The results of the present clearance studies confirm this conclusion. Blood haematocrits either fell slightly or did not change during infusion of amiloride. In addition, the rapid time course of the urinary magnesium-sparing effect and the constancy of GFR and mean arterial blood pressure argue against a contribution from extracellular fluid volume contraction to the observed magnesium-sparing effect of amiloride. Since GFR remained unchanged and plasma magnesium concentration was not consistently altered during infusion of amiloride and percentage ultrafilterable plasma magnesium did not differ significantly between the time-control and amiloride-treated animals, the observed magnesium-sparing effect of amiloride could not be attributed to an amiloride-induced fall in the filtered load of magnesium. Mean arterial blood pressure did not change on infusion of amiloride and there was no difference in plasma aldosterone levels between the time-control group and the amiloride-treated groups.

The urinary conservation of magnesium by amiloride was found to be dose-dependent over the dose range investigated, a finding similar to that for the urinary conservation of potassium, but not for the effect on urinary calcium or sodium excretion. This suggested a specific renal action of amiloride altering both potassium and magnesium transport, that was not consistently related to an effect on urinary sodium excretion.

The magnitude of the effect of amiloride on urinary magnesium excretion was much less than that on potassium excretion. This difference may, to some extent reflect the difference in the magnitude of the contribution of the distal tubule to the renal handling of magnesium and potassium. The conservation of urinary magnesium by amiloride during the frusemide-induced diuresis was more marked than that observed previously in Ringer-loaded rats (Devane *et al.*, 1980).

There are a number of possible explanations why amiloride was a more effective magnesium conserving agent during a frusemide-diuresis: (1) Frusemide has been shown to cause an increased delivery of magnesium to the distal tubule (Quamme, 1981). Reabsorption of magnesium in the distal tubule has been reported to be load-dependent (Quamme & Dirks, 1980). If amiloride acted to promote reabsorption of magnesium in the distal tubule, then a greater effect was likely during a frusemide diuresis which involved an enhanced delivery of magnesium to this site. (2) Frusemide stimulates the secretion of potassium in the distal tubule (Duarte *et al.*, 1971). The potassium-sparing action of amiloride is more marked when amiloride is combined with frusemide (Gussin, 1977). It has been suggested that secretion of magnesium also occurs in the distal tubule (Wen, Evanson & Dirks, 1970). The experimental conditions in which magnesium secretion in the distal tubule has been most consistently observed have involved a frusemide diuresis (Duarte, 1968a; Wen, Wong & Dirks, 1971; Rios, Ingram, Ingram & Di Bona, 1977). If amiloride acts by inhibiting magnesium secretion in the distal tuble, then a more marked effect was likely under conditions of a frusemide-diuresis.

However, further studies are required to determine whether amiloride exerts its magnesium-sparing effect by promoting the reabsorption of magnesium or by inhibiting the secretion of magnesium.

Amiloride also caused a significant reduction in the fractional excretion of calcium. This effect was not dose-dependent. Our previous studies with amiloride in saline-loaded rats also showed a reduced urinary excretion of calcium during a frusemide diuresis but this effect was not observed when amiloride was administered alone (Devane *et al.*, 1981a). An acute hypocalciuric effect of amiloride was also observed in clearance studies in dogs (Costanzo & Weiner, 1976). These authors also observed an acute hypocalciuric effect with chlorothiazide, and suggested that these agents may be promoting a passive reabsorption of calcium, secondary to a reduced transepithelial potential difference in the distal tubule.

This work was supported by the Medical Research Council of Ireland. Amiloride hydrochloride was a gift from Merck, Sharp and Dohme.

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(*Received March 4, 1983.*)