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THE EFFECTS OF AMILORIDE AND TRIAMTERENE ON URINARY MAGNESIUM EXCRETION IN CONSCIOUS SALINE-LOADED RATS

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1 The potassium-sparing diuretics, triamterene and amiloride, reduced urinary magnesium excretion in conscious saline-loaded rats.

2 Urinary magnesium-conservation was also detected when amiloride was used in combination with the potent 'loop-blocking' diuretic, frusemide.

Introduction

One of the serious problems associated with diuretic therapy results from increased urinary potassium excretion during prolonged therapy with potent diuretics. In attempts to prevent this, potassium-sparing diuretics can be administered concomitantly with the more potent diuretics. Large urinary losses of magnesium have also been shown to occur with potent diuretics especially loop-blocking drugs (Duarte, 1968; Ryan & Phillips, 1977). Magnesium deficiency *per se* can result in potassium depletion and experimental studies have shown that magnesium is required for both the maintenance and restoration of cellular potassium (Whang & Welt, 1963; Ryan & Hingerty, 1969). Although, the effects of potassium-sparing diuretics on renal magnesium handling have not been widely studied, there are some indications that these drugs may reduce urinary magnesium excretion. Urinary magnesium excretion was reduced by spironolactone in patients with primary aldosteronism (Horton & Biglieri, 1962) and by canrenoate-potassium in patients with liver cirrhosis (Lim & Jacob, 1978). Triamterene administration to normal subjects (Hanze & Seyberth, 1967) and amiloride administration to congestive heart failure patients (Counihan, Dunne, Halley, Ryan & Ryan, 1978) have also been reported to reduce urinary magnesium excretion.

We have investigated whether potassium-sparing diuretics have magnesium sparing properties when administered (a) alone or, (b) in combination with the potent 'loop-blocking' diuretic frusemide in conscious saline-loaded rats. Some of our findings have been presented in preliminary form (Devane & Ryan, 1979).

Methods

Male Wistar rats were deprived of food overnight but allowed free access to tap water. Food and water were withheld for the duration of the experiment. On the morning of the experiment all animals received a saline-load (2.5 ml/100 g body wt. of a 0.9% w/v NaCl solution) by gastric tube. The diuretic agents were administered as a suspension in the appropriate volume of saline. Each rat was then placed in an individual metabolism cage and urine was collected for 6 h. All experiments were carried out at the same time of day. In each experiment a diuretic-treated group was compared with a control group. Control and diuretic-treated animals were matched for body weight.

Triamterene

In this experiment a saline-loaded control group, weight 185.0 g ± 12.1 (mean ± s.e. mean) was compared with a triamterene-treated group, weight 179.2 g ± 11.0 (mean ± s.e. mean). The dose of triamterene (25 mg/kg body wt.) represents approximately the mid-point in the natriuretic dose-response curve for orally administered triamterene in conscious saline-loaded rats (Wiebelhaus, Weinstock, Maass, Brennan, Sosnowski & Larsen, 1965).

Amiloride

Two groups of rats were investigated. A saline-loaded control group, weight 361.7 g ± 12.7 (mean ± s.e. mean), and a group that had received amiloride hydrochloride (2.5 mg/kg body wt.), weight 348.7 g ± 14.6 (mean ± s.e. mean).

Amiloride plus frusemide

In this experiment two groups of rats were studied. A group which had received frusemide (40 mg/kg body

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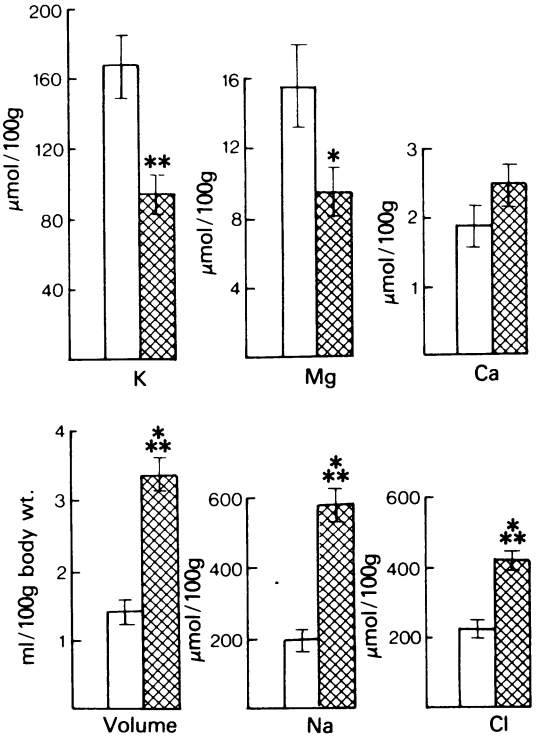

Figure 1 Effect of triamterene (25 mg/kg) on urinary electrolyte excretion in the conscious saline-loaded rat. Urine was collected over a 6 h period in both control (open columns) and triamterene-treated (cross-hatched) animals and the results are expressed as the total urinary output over the 6 h period. Mean values are shown; vertical lines indicate s.e. means; $n = 6$ for control and triamterene-treated rats. $***P < 0.001$; $**P < 0.01$; $*P < 0.05$ for differences in response between control and triamterene-treated animals.

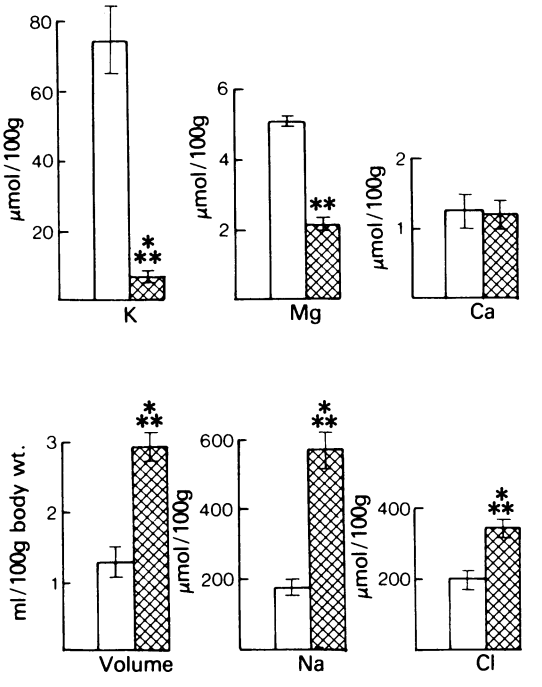

Figure 2 Effect of amiloride (2.5 mg/kg) on urinary electrolyte excretion in the conscious saline-loaded rat. Urine was collected over a 6 h period in both control (open columns) and amiloride-treated (cross-hatched columns) animals, and the results are expressed as the total urinary output over the 6 h period. Mean values are shown; vertical lines indicate s.e. means. $n = 11$ for control and 18 for amiloride-treated rats. $***P < 0.001$; $**P < 0.01$; $*P < 0.05$ for differences in response between control and amiloride-treated animals.

weight), weight 145.2 g ± 18.7 (mean ± s.e. mean) and a group which received amiloride hydrochloride (2.5 mg/kg body wt.) in the presence of the same dose of frusemide, weight 153.8 g ± 15.3 (mean ± s.e. mean). The dose of frusemide represents the mid-point of the natriuretic dose-response curve for orally administered frusemide to conscious rats. (Timmerman, Springman & Thoms, 1964).

Analysis of urine

The urine was analysed for sodium and potassium by flame emission spectrophotometry and for magnesium and calcium concentrations by atomic absorption spectrophotometry, using the Pye Unicam SP90A spectrophotometer in each case. Urinary chloride was measured by the Cotlove-type silver electrode potentiometric method, using an Eel chloride meter.

Drugs

The drugs used were: triamterene B.P. (Smith, Kline and French), amiloride hydrochloride, B.P. (Merck, Sharp and Dohme) and frusemide, B.P. (Hoechst).

Results

Triamterene (Figure 1) caused a significant diuresis ($P < 0.001$). Sodium excretion was significantly increased ($P < 0.001$). The triamterene group excreted an average of 150% of the administered saline-load compared with an average of 50% by the control group. Chloride excretion was also increased significantly ($P < 0.001$) but to a lesser extent than sodium excretion. Triamterene significantly reduced the urinary output of potassium ($P < 0.01$) and magnesium ($P < 0.05$). No significant change was noted in calcium excretion.

The results of oral administration of amiloride to saline-loaded rats are indicated in Figure 2. Amiloride

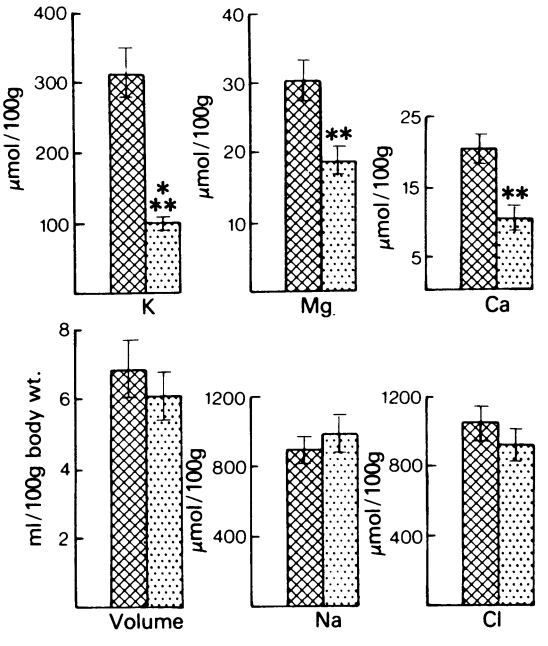

Figure 3 Effects of frusemide alone (40 mg/kg) and frusemide (40 mg/kg) plus amiloride (2.5 mg/kg) on urinary electrolyte excretion in conscious saline-loaded rats. Urine was collected over a 6 h period in both groups of animals, and results are expressed as the total urinary output over the 6 h period. Cross-hatched columns, frusemide alone; stippled columns, frusemide plus amiloride. Mean values are shown; vertical lines indicate s.e. means. $n = 6$ for frusemide alone and 5 for frusemide plus amiloride. $^{***}P < 0.001$; $^{**}P < 0.01$; $^{*}P < 0.05$ for differences in response between frusemide and frusemide plus amiloride-treated animals.

caused a significant diuresis ($P < 0.001$) and also promoted the excretion of sodium ($P < 0.001$). The amiloride group excreted an average of 148% of the administered saline-load compared with 45% by the control group. The urinary excretion of potassium ($P < 0.001$) and magnesium ($P < 0.01$) decreased significantly following amiloride administration. Calcium excretion was not significantly altered.

The effects of amiloride in the presence of frusemide are shown in Figure 3. Amiloride in the presence of frusemide caused no further increase in urinary volume, sodium or chloride excretion, over that seen in the presence of frusemide alone. The frusemide group excreted an average of 235% of the administered saline-load and the amiloride plus frusemide group excreted an average of 258%. However amiloride in the presence of frusemide did cause a significant fall in the urinary excretion of potassium ($P < 0.001$), magnesium ($P < 0.01$) and calcium ($P < 0.01$).

Discussion

These results provide further evidence that potassium-sparing diuretics can, under certain conditions, reduce urinary magnesium excretion. These findings may help in understanding mechanisms of magnesium handling by the kidney. Micropuncture studies in rats indicate that approximately 30% of filtered magnesium is reabsorbed in the proximal tubule (Brunette, Vigneault & Carriere, 1974). Magnesium concentration in tubular fluid increased along the proximal tubule indicating dissociation from reabsorption of sodium and fluid. The micropuncture data indicate that the loop of Henle reabsorbs the major portion of magnesium accounting for approximately 55% of the filtered magnesium (Brunette *et al.*, 1974). In both the saline-loaded rat (Brunette *et al.*, 1974) and saline-loaded dog (Wen, Evanson & Dirks, 1970), micropuncture studies suggest only a minor role for the distal tubule in the reabsorption of magnesium and accounting for only 5% of the filtered magnesium.

Although the mechanism(s) by which triamterene and amiloride reduced urinary magnesium excretion in our studies is not clear, a number of possibilities can be suggested.

(1) Enhanced magnesium absorption from the distal convoluted tubule and collecting duct. Triamterene and amiloride are believed to reduce urinary potassium excretion primarily by an action on sodium transport in the distal tubule and collecting duct (Seely & Dirks, 1977). Amiloride, by reducing luminal sodium permeability, lowers the transepithelial electrical potential (Duarte, Chomety & Giebish, 1971). Such a reduction in transtubular negativity might be expected to favour reabsorption of magnesium ions.

(2) Blockade of magnesium secretion in the distal tubule and collecting duct. The question of magnesium secretion by renal tubules is somewhat controversial. Averill & Heaton (1966) found magnesium secretion in rat kidneys during magnesium loading. However, other workers could not confirm magnesium secretion in rats (Alfredson & Walser, 1970) or dogs (Massry, Coburn & Kleemen, 1969). Magnesium secretion has also been reported during saline and frusemide diuresis (Duarte, 1968; Wen, Wong & Dirks, 1971; Rios, Ingram, Ingram & Di Bona 1977) and in magnesium-loaded phosphate-depleted rats (Sachtjen, Meyer & Massry, 1979). Lechene & Blouch (1979) have detected magnesium and potassium secretion in the cortical collecting ducts during saline diuresis in rats. It is conceivable that under the experimental conditions employed in our studies, magnesium secretion did occur and that amiloride had an inhibitory effect on magnesium secretion. However, further, more direct studies would be required to test this hypothesis.

(3) Enhanced magnesium reabsorption from the renal proximal tubule in response to possible extracellular fluid volume contraction resulting from diuresis. Both the triamterene- and amiloride-treated groups excreted approximately 150% of the administered saline load suggesting the possibility of extracellular volume contraction. Frusemide resulted in a natriuresis equivalent to 235% of the administered saline load, a level likely to involve depression of the extracellular fluid volume. The fact that amiloride in the presence of frusemide failed to produce any additional natriuresis while reducing urinary potassium also suggests extracellular volume contraction with a consequent enhancement of sodium and fluid reabsorption from the proximal tubule. This could lead to additional net magnesium reabsorption from the proximal tubule even though magnesium reabsorption in this area is dissociated to some degree from sodium and fluid reabsorption.

Although the doses used in this study are much higher than those normally used clinically (triamterene 150 to 250 mg daily, amiloride 5 to 20 mg daily, frusemide 40 mg daily), they are appropriate to the experimental model used. The dose of triamterene (25 mg/kg) represents the mid-point in the natriuretic dose-response curve for orally-administered triamterene in conscious saline-loaded rats (Wiebelhaus *et al.*, 1965). The dose of amiloride (2.5 mg/kg) was chosen as one producing a similar level of natriuresis to the triamterene dose. The dose of frusemide (40 mg/kg) represents the mid-point of the natriuretic dose-response curve for orally administered frusemide in conscious rats (Timmerman *et al.*, 1964). Further studies are required to establish whether potassium-sparing diuretics can reduce magnesium excretion in clinically more appropriate situations.

Magnesium conservation by the potassium-sparing diuretics triamterene and amiloride could be a useful effect of these drugs. Factors predisposing to potassium loss, such as potent diuretic therapy and aldosteronism also enhance loss of magnesium. Potassium and magnesium deficiencies are likely to co-exist in many situations. Magnesium has been shown in experimental animals to be required for the maintenance and restoration of cell potassium (Whang & Welt, 1963; Ryan & Hingerty, 1969). Indeed, co-existing magnesium deficiency may be a common cause of refractoriness to potassium repletion with potassium supplements (Whang & Aikawa, 1977).

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