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Mechanism of Negative Potassium Balance in the Magnesium-Deficient Rat (41291)

LINDA L. FRANCISCO, LINDA L. SAWIN, AND GERALD F. DIBONA
With the technical assistance of Mary Jo Ingram and F. Duane Ingram

Department of Internal Medicine, University of Iowa, College of Medicine and Veterans Administration Medical Center, Iowa City, Iowa 52242

Abstract. Magnesium (Mg) deprivation leads to an increased urinary potassium excretion (U_KV) and a decreased plasma potassium (P_K) concentration. To evaluate the role of aldosterone in this process, rats were placed on a low-Mg diet (0.3 mg Mg/g diet). Group I animals received the low-Mg diet supplemented with normal Mg intake (2.6 mg Mg/g diet) while Group II animals received the low-Mg diet alone. Group III animals received the low-Mg diet plus spironolactone (10 mg/kg/day, sc). After 16 days on their respective diets, P_K was higher (4.4 ± 0.1 vs 3.6 ± 0.1 mEq/liter, $P < 0.001$), cumulative K balance was more positive (1.63 ± 0.31 vs −0.24 ± 0.16 mEq, $P < 0.001$), and plasma aldosterone concentration was lower (150 ± 16 vs 241 ± 15 ng/dl, $P < 0.001$) in Group I animals compared to Group II animals. P_K was returned toward control (3.9 ± 0.2 mEq/liter), positive K balance was restored (1.94 ± 0.25 mEq), while plasma aldosterone concentration remained elevated (280 ± 33 ng/dl) in Group III animals treated with spironolactone. Micropuncture studies revealed a distal tubular site for K entry in Group II animals as compared to Group I animals; this was reversed in Group III animals. It is concluded that dietary Mg deprivation produces kaliuresis, a fall in P_K and a rise in plasma aldosterone concentration. The kaliuresis and negative K balance are reversed by the administration of spironolactone. Micropuncture analysis demonstrated that the kaliuresis was due to increased K entry along the distal tubule consistent with the site of action of aldosterone.

The contribution of the kidney to the syndrome of magnesium (Mg) deprivation secondary to dietary Mg restriction has been studied in man (1). It has been demonstrated that urinary Mg excretion falls to extremely low levels in the first 72 hr of dietary Mg deprivation. This fall in urinary Mg excretion is accompanied by a decrease in urinary sodium (Na) excretion and an increase in urinary potassium (K) excretion despite the presence of hypokalemia. This pattern of urinary electrolyte excretion is suggestive of enhanced distal Na and Mg reabsorption coupled with K excretion. The reciprocal relationship between Na and K excretion has suggested a role for aldosterone in the renal electrolyte handling seen in dietary magnesium deprivation.

A similar pattern of urinary electrolyte excretion is seen in the dog (2) and rat (3) maintained on a Mg-deficient diet. Plasma aldosterone concentrations have been measured in both the Mg-deprived dog (2) and the rat (4) and have been demonstrated to be elevated lending support to the thesis that the kaliuresis and subsequent hypokalemia observed in Mg deficiency may be secondary to the effect of aldosterone on the distal nephron to promote K excretion. This study was designed to specifically examine the location along the nephron of enhanced urinary K excretion in dietary Mg deprivation and to evaluate the role of aldosterone in this process.

Materials and Methods. Twelve- to fourteen week-old female Sprague–Dawley rats were placed into one of three groups and kept in individual metabolic cages for daily measurements of dietary intake and urinary output. Group I ($N = 8$) animals received a low-Mg diet (Na = 0.260 mEq/g dry wt, K = 0.046 mEq/g dry wt, Mg = 0.025 mEq/g dry wt or 0.3 mg Mg^{2+}/g dry wt, Nutritional Biochemical Corp., Cleveland, Ohio), that was supplemented to a normal Mg content with 2.6 mg Mg^{2+}/g dry wt diet as $MgCl_2 \cdot 6H_2O$. Group II ($N = 8$) animals received the low-Mg diet alone. Group III ($N = 8$) animals received the low-Mg diet and daily injections of spi-

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ronolactone (Sigma Chemical, St. Louis, Mo.), 10 mg/kg/day sc, that was begun on Day 5 of the protocol. The animals were pair fed and allowed free access to demineralized water. Between Days 18 and 25 of the balance study, animals from each dietary treatment group were anesthetized with sodium pentobarbital (50 mg/ml), 50 mg/kg administered intraperitoneally, and placed on a thermostatically regulated heated micropuncture table. The left kidney was exposed through a left subcostal incision and after dissection from the surrounding tissue, was mounted in a Lucite chamber. Care was taken to avoid compression of the vascular pedicle, visible assessment of the amplitude and frequency of renal pulsations and of capillary flow served as control criteria. The kidney surface was continuously bathed with 38° mineral oil and illuminated with a fiberoptic light source. The kidney was not decapsulated. Catheters (PE 50 or greater) were placed in the left carotid artery, right and left jugular veins, and the left ureter for measurement of arterial pressure, infusion of solutions and of F, D, and C, Green No. 3 dye, and urine collections. Arterial pressures were monitored by an electronic transducer (Statham P23Db, Statham Instruments, Inc., Oxnard, Calif.) connected to a direct writing recorder (Beckman Dynagraph R411, Beckman Instruments, Inc., Schiller Park, Ill.). Exposed tissues were covered with gauze pads moistened with 38° 0.9% NaCl. An infusion of 0.9% NaCl was established at the time of right jugular vein catheterization and maintained at 0.05 ml/min thereafter. At the end of surgery, proximal tubular transit time was determined by the method of Steinhausen (5) following a left jugular vein injection of 0.05 ml of 5% aqueous (distilled water) solution of F, D, and C, Green No. 3 dye (pH 7.0). Animals whose transit times were in excess of 12 sec were not used.

Each animal received a priming dose of 200 mg/kg inulin in 1.5 ml of 0.9% NaCl and 6 mg/kg/min was added to the sustaining infusion of 0.9% NaCl at 0.05 ml/min. During the subsequent 60-min equilibration period, late proximal (PCT), early distal (DCT), and late distal (DCT) tubular segments were identified (5) with an intravenous injection of F, D, and C, Green No. 3 dye.

During the experimental period, two 30-min urine collections were made into preweighed containers under oil. Fifty to one hundred microliters of arterial blood samples was taken at the midpoints of the urine collections. Timed tubular fluid collections were made from late PCT and early and late DCT sites with sharpened micropipets of 8- to 10-μm tip diameter. An antegrade Sudan black-stained oil block of 4–6 tubular diameter was introduced into the tubular lumen and tubular fluid was collected spontaneously with gentle aspiration so as to maintain tubular diameter relatively constant. Collection times ranged from 2 to 8 min. At the end of each collection, the micropipet tip was sealed with clear oil to prevent sample evaporation. In 10 Group I animals, tubular fluid samples were taken from 26 late PCT, 21 early DCT, and 24 late DCT sites. In 12 Group II animals, tubular fluid samples were taken from 30 late PCT, 30 early DCT, and 18 late DCT sites. In 6 Group III animals, tubular fluid samples were taken from 21 late PCT, 18 early DCT, and 10 late DCT sites. Prior to sacrifice, the animal was bled from the carotid artery into a heparinized syringe and the plasma frozen for measurement of plasma Na, K, Mg, and aldosterone concentrations.

Analytical methods. Inulin was measured in plasma, urine, and tubular fluid by the method of Vurek and Pegram (6). Sodium and potassium were measured in plasma and urine by flame photometry (Instrumentation Laboratory, Inc., Lexington, Mass.). Magnesium was measured in plasma and urine by colorimetric method of Gitelman *et al.* (7). Sodium and potassium were measured in tubular fluid by electron probe microanalysis (8, 9). Plasma aldosterone concentrations were measured by radioimmunoassay (New England Nuclear, North Billerica, Mass.).

Calculations. The urinary clearance of inulin from plasma was taken as a measure of glomerular filtration rate (GFR). Daily K balance was taken as the difference between daily dietary K intake and daily uri-

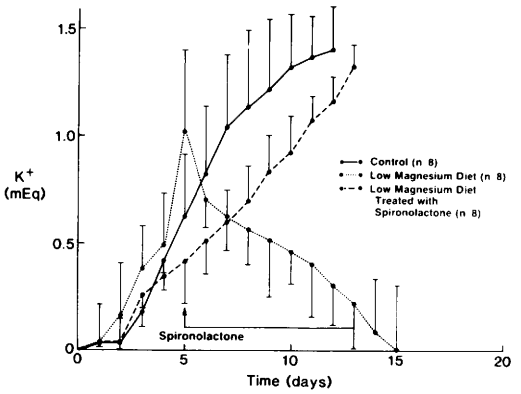

FIG. 1. Cumulative K balance, calculated as the sum of daily K balance with daily K balance = daily dietary intake of K − daily urinary excretion of K, is plotted on the ordinate with time in days on the diet plotted on the abscissa. Fecal K was not included in the calculations but there was no evidence of excess K loss via diarrhea in any of the three groups of animals. Each point on the curve is the mean ± SE.

nary K excretion. The data in the text, figures, and tables are presented as mean ± SE. Students' *t* test was used for statistical analysis between each group.

Results. Figure 1 presents the results of the balance portion of the study with cumulative K balance expressed in milliequivalents. Group I, control animals, were in daily positive K balance throughout the entire period of study. Group II animals receiving the low-Mg diet, were in positive daily K balance until Day 5 at which time urinary output of K exceeded dietary intake. By Day 15, these animals returned to zero K balance. Though not shown on this figure, beyond Day 15, Group II animals continued to have U_KV that exceeded the dietary intake of K, resulting in negative daily and cumulative K balance. Group III animals receiving daily injections of spironolactone beginning on Day 5 were in positive K balance throughout the entire period of the study.

Table I presents data obtained at the time of the renal micropuncture and clearance studies. Plasma Mg and K concentrations were significantly lower, fractional excretion of potassium (FE_K) was greater, and plasma aldosterone concentration higher in the animals on the low-Mg diet. Spironolactone therapy increased plasma K concentration and decreased FE_K in the Group III animals. Although the FE_K in spironolactone-treated Group III animals was decreased significantly below that of Group I control animals, the plasma K concentration was only partially restored to the control level. This may reflect the fact that the plasma K concentration represents the cumulative effect of 2 to 3 weeks of dietary and pharmacological manipulation in conscious animals whereas the measurement of FE_K is a single point determination in anesthetized surgically operated animals.

Standard curves for electron probe measurement of the two cations, Na and K, were prepared from measurements on standards consisting of aqueous solutions each containing Na and K as their chloride salts in concentrations encompassing the phys-

TABLE I

	Group I Control	Group II Low magnesium diet	Group III Low magnesium treated with spironolactone
N	10	12	6
Body weight (g)	207 ± 23	221 ± 27	246 ± 33
GFR (ml/min)	0.80 ± 0.11	0.77 ± 0.07	0.68 ± 0.08
Plasma Mg (mEq/L)	1.92 ± 0.14	0.63 ± 0.04**	0.47 ± 0.05**
Plasma K (mEq/L)	4.4 ± 0.1	3.6 ± 0.1**	3.9 ± 0.2
FE_K (%)	14 ± 1	27 ± 2*	6 ± 2
FE_{Mg} (%)	11 ± 1	1 ± 0.1	5 ± 2
Plasma aldosterone (ng%)	150 ± 16	241 ± 15**	280 ± 33**

Note. Data are mean ± SE.
* $P < 0.05$ compared to Group I. ** $P < 0.001$ compared to Group I.

iologic range of interest. A 30-pl sample of each standard solution was analyzed for both cations. The energy of the electron probe beam was set at 15 kV with a current of 200 nA for Na and 300 nA for K, and a width of 50 μm for both cations. Total counting time per sample was 100 sec for Na and 50 sec for K. For Na, the minimum detectable concentration was 0.8 mmol/liter with a signal-to-background ratio of 22.73 at 20 mmol/liter. The relationship between counts per 10 sec (Y) and Na concentration in the standard (X) was linear with the regression equation being $Y = 20.3\,X + 0.87$ and the correlation coefficient, r, being 0.99. For K, the minimum detectable concentration was 0.25 mmol/liter with a signal-to-background ratio of 5.02 at 25 mmol/liter. The relationship between counts per 10 sec (Y) and K concentration in the standard (X) was linear with the regression equation being $Y = 13.72\,X + 1.15$ and the correlation coefficient, r, being 0.98.

The results of the micropuncture analysis are shown in Fig. 2 and Table II for the three groups of animals. The data represent the mean ± SE of the average values in each animal. In the upper panel of Fig. 2, fractional excretion of Na along the entire nephron was not markedly different among the three groups of animals. The lower panel shows the handling of K by the nephron. There was an increase in the fractional rejection of K in the proximal tubule in the Group II animals as compared to Group I animals (0.66 ± 0.07 vs 0.53 ± 0.08, $P < 0.02$). This increase in fractional rejection was reversed when the animals were treated with spironolactone, Group III (0.35 ± 0.04). By the early DCT, there was from 14% (Group II) to 31% (Group I) of the filtered K remaining in the tubular lumen. Along the DCT between the early and late distal punctures, there was K entry into the tubular lumen in Group II, Mg-depleted animals as compared to control, Group I animals. This increase in K in the tubular fluid persisted into the final ureteral urine, resulting in the increase in the fractional excretion of K in the Group II animals as compared to Group I. Spironolactone prevented the K entry into the distal portion of the nephron and thus prevented the kaliuresis.

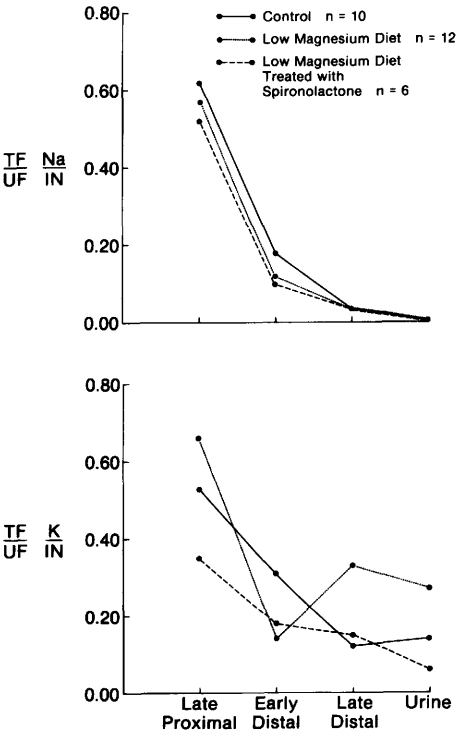

FIG. 2. Micropuncture data for the three groups of animals are shown as the mean of the average values in each animal. The control animals are shown in the solid lines, the animals on the low-magnesium diet are shown in the dotted lines, and the animals on the low-magnesium diet treated with spironolactone are shown in the dashed lines. The tubular fluid (TF)-to-ultrafilterable (UF) ratio of sodium (Na) to inulin (IN) is shown in the upper panel, and TF/UF ratio of K to IN is shown in the lower panel. Late proximal, early distal, and late distal micropuncture sampling sites of superficial nephrons and final ureteral urine are shown on the abscissa.

Discussion. These studies have demonstrated that dietary Mg deprivation produces hypomagnesemia, a significant fall in plasma K concentration, an increase in U_KV, and an elevation of plasma aldosterone concentration. This kaliuresis and subsequent negative K balance are reversed by the administration of a selective competitive inhibitor of aldosterone. Micropuncture data located the site of entry of K into the tubular fluid to the distal portion

TABLE II. MICROPUNCTURE ANALYSIS

		Late proximal						Early distal					
		I	II	III	I vs II	II vs III	I vs III	I	II	III	I vs II	II vs III	I vs III
$\frac{TF}{UF}$	IN[a]	1.90 ± 0.18	1.92 ± 0.10	1.96 ± 0.10	NS	NS	NS	3.38 ± 0.21	3.29 ± 0.20	4.27 ± 0.51	NS	<0.05	NS
$\frac{TF}{UF}$	Na	1.06 ± 0.07	1.04 ± 0.03	1.00 ± 0.04	NS	NS	NS	0.58 ± 0.10	0.35 ± 0.02	0.40 ± 0.03	<0.05	NS	NS
$\frac{TF}{UF}$	$\frac{Na}{IN}$	0.62 ± 0.06	0.57 ± 0.03	0.52 ± 0.02	NS	NS	NS	0.18 ± 0.03	0.12 ± 0.01	0.10 ± 0.01	<0.05	NS	<0.05
$\frac{TF}{UF}$	K	0.96 ± 0.16	1.22 ± 0.07	0.71 ± 0.10	NS	<0.001	NS	1.01 ± 0.20	0.48 ± 0.06	0.70 ± 0.23	<0.05	NS	NS
$\frac{TF}{UF}$	$\frac{K}{IN}$	0.53 ± 0.08	0.66 ± 0.07	0.35 ± 0.04	<0.02	<0.005	<0.05	0.31 ± 0.07	0.14 ± 0.01	0.18 ± 0.06	<0.05	NS	NS

		Late distal						Urine					
		I	II	III	I vs II	II vs III	I vs III	I	II	III	I vs II	II vs III	I vs III
$\frac{TF}{UF}$	IN[a]	9.43 ± 1.21	6.75 ± 0.43	8.66 ± 1.02	<0.05	NS	NS	122.13 ± 19.82	192.92 ± 22.91	174.14 ± 23.18	<0.05	NS	NS
$\frac{TF}{UF}$	Na	0.29 ± 0.03	0.23 ± 0.03	0.20 ± 0.03	NS	NS	<0.05	0.83 ± 0.09	0.71 ± 0.11	0.59 ± 0.11	NS	NS	NS
$\frac{TF}{UF}$	$\frac{Na}{IN}$	0.04 ± 0.01	0.04 ± 0.01	0.03 ± 0.001	NS	NS	NS	0.01 ± 0.001	0.01 ± 0.001	0.01 ± 0.002	NS	NS	NS
$\frac{TF}{UF}$	K	1.21 ± 0.23	2.28 ± 0.41	1.03 ± 0.17	<0.05	<0.01	NS	18.68 ± 3.18	46.60 ± 4.01	8.39 ± 2.58	<0.001	<0.001	<0.05
$\frac{TF}{UF}$	$\frac{K}{IN}$	0.12 ± 0.01	0.33 ± 0.05	0.15 ± 0.04	<0.001	<0.01	NS	0.14 ± 0.01	0.27 ± 0.02	0.06 ± 0.02	<0.001	<0.001	<0.01

Note. The data represent the mean ± SE of the average values in each animal.
[a] TF, tubular fluid; UF, ultrafilterable; Na, sodium; IN, inulin; K, potassium.

of the nephron consistent with the site of action of aldosterone.

The observation that dietary deprivation of Mg in the rat and man is accompanied by kaliuresis and subsequent muscle K deficits is well established (10, 11). In addition, several investigators have reported alterations in plasma levels or secretion rates of adrenocortical hormones in Mg-deficient animals (2, 4, 12, 13). In general, corticosterone secretion and plasma corticosterone concentration were found somewhat below control values in Mg deficiency (4, 12, 14). Aldosterone secretion was reported to be elevated in rats on Day 55 of Mg depletion by Ginn *et al.* (4). In a morphological study, Cantin found widening of the zona glomerulosa of the adrenal cortex suggesting increased production of aldosterone in Mg deficiency as compared to control (3). Our results confirm these findings and establish elevated plasma concentrations of aldosterone in the rats by Days 18–25 of Mg deprivation. In addition, we have demonstrated functional evidence for excess aldosterone secretion in Mg deprivation with increased K entry into the distal tubular lumen and subsequent kaliuresis that is reversed by administration of an aldosterone antagonist, spironolactone.

The mechanism for this increase in aldosterone secretion is not established as yet. Mg deficiency could directly stimulate the zona glomerulosa of the adrenal cortex to synthesize and secrete aldosterone or there could be an effect of Mg deficiency to directly stimulate the juxtaglomerular apparatus to secrete renin with a secondary increase in aldosterone secretion. Less likely, there could be a decrease in the degradation of aldosterone in the Mg-deficient state. Cantin has shown an increase in the juxtaglomerular index in the Mg-deficient rat as compared to control suggesting stimulation of the renin–angiotensin system with a secondary hyperaldosteronism (3). Helber *et al.*, in the dog, found an increase in aldosterone secretion; however, there was no increase in the renin–angiotensin system as evaluated by plasma renin activity (2). The rise in aldosterone secretion that they observed was prevented when Mg concentration in the isolated adrenal blood supply was increased 30–50%.

The dietary intake employed in this study likely affected the renal tubular handling of both ions examined. The sodium content of the diet was 0.260 mEq/g dry wt which is approximately 30% higher than that found in normal commercial rat diet (0.200 mEq/g dry wt). In addition, the rats were infused with 0.9% NaCl at 0.05 ml/min, a higher infusion rate than often employed (0.02 ml/min). Thus, the rats had an increased sodium intake, chronic dietary and acute intravenous, which might be responsible for the slightly increased DCT (TF/UF) K/In seen in Group I. During saline loading, Malnic *et al.* (15) noted a mean early DCT (TF/UF) K/In of 0.27 at a mean (TF/UF) In of 3.38; in Group I, mean early DCT (TF/UF) K/In was 0.31 at a mean (TF/UF) In of 3.38. In addition, the potassium content of the diet was 0.046 mEq/g dry wt which is approximately 77% less than that found in normal commercial rat diet (0.200 mEq/g dry wt). Malnic *et al.* (14) noted K reabsorption along the DCT in rats placed on a low-potassium diet. Our findings in Group I animals are similar in that K reabsorption continued along the DCT. These observations make our finding of K entry along the DCT in Group II animals even more striking and, with reversal of this phenomenon by spironolactone in Group III animals, focuses on the DCT as the site of renal tubular K wasting in the kaliuresis of Mg deprivation.

It is known that Mg depletion leads to hypercalcemia, hyperphosphatemia, and increase parathormone levels in rats (16, 17). Although these variables were not determined in this study it is possible that they, acting singly or in combination, could have played a role in the altered renal tubular handling of potassium we observed. However, if that were the case, it would be somewhat difficult to explain the prompt and complete reversal of renal tubular potassium handling to normal by spironolactone.

In summary, dietary Mg deprivation in the rat produces renal potassium wasting which leads to negative potassium balance and hypokalemia and is associated with in-

creased plasma aldosterone concentrations. The renal potassium wasting was localized by micropuncture to increased entry of potassium into the distal convoluted tubule lumen. Treatment with spironolactone normalizes the urinary potassium excretion, the potassium balance, the serum potassium concentration and decreases the entry of potassium into the distal convoluted tubule lumen. The findings are consistent with the hypothesis that the abnormalities of potassium metabolism observed in the magnesium-deficient rat are due to a state of hyperaldosteronism.

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