Phosphaturia in magnesium-deficient rats

H. EARL GINN,3 AND LINDA L. SHANBOUR

Departments of Medicine, Physiology, and Urology, Vanderbilt University Medical School, Nashville, Tennessee; and Department of Physiology, University of Alabama, Birmingham, Alabama


The effect of magnesium deficiency on urinary phosphate excretion was studied in rats rendered magnesium deficient by diet. Increased urinary phosphate excretion was observed. Tubular maximum reabsorption (Tm) of inorganic phosphate was lower in magnesium-deficient (0.9 ± 0.2 μM/ml per min) than control animals (1.0 ± 0.3 μM/ml per min, P < .001). Since hypomagnesemia was accompanied by hypercalcemia, Tm of inorganic phosphate was measured in parathyroidectomized rats. In this group the low-magnesium animals had a Tm of 1.0 ± 0.8 μM/ml per min compared to 4.3 ± 0.5 μM/ml per min in controls, P < .001. It is concluded that the phosphaturia of hypomagnesemia is not due solely to hyperparathyroidism. The responsible mechanism remains elusive.

magnesium deprivation, phosphorus; parathyroidectomy; decreased Tm phosphate

ONE OF THE ALTERATIONS observed in magnesium-deficient rats is their augmented urinary phosphate excretion (31, 37). The present study was undertaken to ascertain the effects of magnesium deficiency on renal tubular phosphate reabsorption. Whether the phosphaturia was related to hyperfunctioning parathyroid glands was also investigated since hypomagnesemic rats have been found to have an associated hypercalcemia (9, 31).

METHODS

Experiment A. The experimental animals, initially weighing 80-100 g, were made magnesium deficient by a diet (31) which contained 0.6 mg magnesium/100 g diet but had all other known essential dietary constituents in adequate amounts. The control rats were pair fed the same diet except for the addition of 50 mg magnesium as magnesium chloride/100 g diet. All animals were permitted demineralized drinking water ad lib. By use of specially constructed individual metabolic cages, 24-hr feces-free urine collections were made each day during the 5th week of study and phosphate excretion determined.

To study renal tubular phosphate reabsorption, after the rats had been on the diet for 6 weeks, solutions containing sodium phosphate buffered to pH 7.4 were infused into a lateral tail vein at a rate of from 0 to 0.025 mM/min to obtain various loads of filtered phosphate. Carbon 14 carboxyl-labeled inulin (from Volk Radio Chemical Co., 803 N. Lake St., Burbank, Calif.), in 0.45% sodium chloride, was added to the phosphate solutions and radioactivity counts were used to measure glomerular filtration rate. Following a 60-min equilibration period, spontaneously voided timed urine collections were made every 15-30 min. Urine flow rates of 0.2-0.3 ml/min were maintained. Blood samples were collected in heparinized capillary tubes from a leg vein before and after each urine collection period for carbon 14 radioactivity counting and for plasma phosphate concentration. The method used for infusions, blood sampling, and urine collections was that of Cotlove (9).

Experiment B. Utilizing a dissecting microscope, surgical removal of the parathyroid glands was performed on another group of 10 magnesium-deficient and 10 control rats after they had been on the diet for 5-6 weeks. Parathyroidectomy was considered successful if plasma calcium concentration was more than two standard deviations below the nonparathyroidectomized groups. Six to nine days following parathyroidectomy, tubular phosphate reabsorption was studied by the method described above in the six pairs of animals that met our criteria for successful parathyroidectomy.

Inorganic phosphate was analyzed by the method of Chen, Toribara, and Warner (8), calcium by the method of Bachra, Dauer, and Sobel (3), and magnesium by the fluorometric method of Schachter (29). Carbon 14 radioactivity was counted by a gas-flow counter after drying 50 μl of the urine or plasma samples mixed with 0.5 ml 0.2% Triton in aluminum planchets. All determinations were performed in duplicate. The average of the plasma values obtained before and after the urine periods was

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TABLE 1. Plasma concentrations of magnesium, calcium, and phosphorus and inulin clearance of control and test animals in experiments A and B

<table>
<thead>
<tr>
<th>Plasma Concen</th>
<th>C14, ml/min</th>
<th>Wt, g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mg, mEq/liter</td>
<td>Ca, mEq/liter</td>
<td>Phos, mM</td>
</tr>
<tr>
<td>Control</td>
<td>Deficient</td>
<td>Control</td>
</tr>
<tr>
<td>1.08±.14</td>
<td>.74±.23*</td>
<td>4.81±.17</td>
</tr>
<tr>
<td>1.81±.58</td>
<td>.80±.08*</td>
<td>3.95±.55</td>
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Values are means ± sd. * P < .001.

used in the calculations. Statistical analyses were performed by the methods of Snedecor (32).

RESULTS

Experiment A. Urinary phosphate excretion during the 5th and 6th weeks of study was 111 ± 29 μM/day in magnesium-deficient animals and 10 ± 5 μM/day in their controls (P < .001). The plasma concentrations of magnesium, calcium, and phosphate in the test and control animals prior to the phosphate infusion studies are listed in Table 1. Plasma phosphate concentrations were not significantly different in the two groups but tended to be lower in the deficient animals. The mean inulin clearances were 2.13 ± 0.45 ml/min in test animals compared to 3.24 ± 0.57 ml/min in controls (P < .001).

Calculations of the tubular phosphate reabsorption when performed at endogenous phosphate levels and expressed as percent of the filtered phosphate load (1-(UPO4)/C14 × PPO4) X 100) gave values of 78.4 ± 6.2% for magnesium-deficient and 94.8 ± 4.8% for control animals (P < .001).

Figure 1 illustrates the filtered phosphate load (C14 × PPO4) plotted on the abscissa and the tubular phosphate reabsorption (C14 × PPO4 − UPO4) V) on the ordinate. The mean maximum tubular reabsorption of phosphate in magnesium-depleted rats, represented by the plateau of the dashed line in Fig. 1, was 2.1 ± 0.4 μM/min compared to 6.0 ± 0.9 μM/min in controls, represented by the solid line. When the maximum tubular phosphate reabsorptions were calculated per milliliter of inulin clearance the test animals had a Tm of 0.9 ± 0.2 μM/ml per min compared to 4.3 ± 0.5 μM/ml per min in controls, P < .001.

Experiment B. Plasma concentrations of magnesium, calcium, and inorganic phosphorus obtained prior to phosphate infusions in the parathyroidectomized rats are listed in Table 1. The calcium concentrations were at least two standard deviations below the nonparathyroidectomized groups, but the hypomagnesemic animals had higher plasma calcium concentrations (4.14 ± .11 mEq/liter) than their controls (3.25 ± .55 mEq/liter), P < .001. The serum inorganic phosphorus levels were not significantly different.

The mean maximum tubular reabsorption of phosphate in parathyroidectomized magnesium-deficient animals was 3.9 ± 1.7 μM/min, represented by the dashed line in Fig. 2, and in their controls was 11.2 ± 0.5 μmoles/min, depicted by the solid line in Fig. 2. When expressed per unit of inulin clearance, the test animals had a Tm of 1.9 ± 0.8 μM/ml per min, compared to 4.3 ± 0.5 μM/ml per min in controls, P < .001.

DISCUSSION

Most of the phosphorus lost from the body appears in the urine. Alterations in phosphorus metabolism in disease states (34) and variations in dietary phosphorus intake (13, 36) are usually accompanied by changes in renal phosphate excretion. The renal mechanism involved in inorganic phosphate ion (mono- and dihydrogen phosphate) excretion include filtration, tubular reabsorption, and possibly tubular secretion (6, 7, 34). Evidence suggests that the urinary excretion of phosphorus is modified by parathyroid hormone (1, 14-16), adrenal cortical hormones (28), vitamin D (10), growth hormone (24), filtered glucose load (27), triiodothyronine (4, 5), and the hydrogen ion status (22, 30).

The experiments herein reported confirm previous studies (31, 37) which showed augmented phosphate excretion in rats on a low-magnesium diet. Our finding tubular maximum kinetics for phosphate reabsorption in the rat kidney differs from the results of Crawford, Gribetz, and Talbot (10), but is in agreement with the recent micropuncture results of Strickler and Thompson (33). The former experiments (10) were performed on parathyroidectomized rats given oral phosphate loads and the estimated quantities of filtered phosphate were lower than those obtained in our studies. It is possible, therefore, that their results were obtained at levels below the tubular maximum.

The magnesium-depleted animals in our experiments had markedly reduced tubular maxima for phosphate reabsorption. These calculations were based on the assumption that urinary phosphate excretion is equal to the nonreabsorbed fraction of filtered phosphate. Although the possibility of augmented tubular secretion of phosphate (6, 7) cannot be excluded in our experiments, the
micropuncture results of Strickler and Thompson (33) suggest absence of phosphate secretion by the rat kidney.

The mechanism responsible for the phosphoruresis is elusive. It is apparently not due to a major catabolic influence, as Whang and Welt (37) found no difference in the urinary excretion of nitrogen in magnesium-depleted and control rats. Increased concentration of corticosterone has been shown to produce phosphoruresis (14). That the phosphoruresis is not related to increased secretion of this steroid, however, is supported by data which showed no increased corticosterone secretion in magnesium-depleted rats (12). Blood from the left main adrenal vein, in the latter studies, was collected over a 10-min period after ligation of the accessory adrenal veins. Corticosterone was analyzed by a microadaptation of the isotope dilution method of Peterson (26). The magnesium-deficient animals had a secretion rate of 0.60 ± 0.104 μg/100 g per min compared to 0.80 ± 0.150 μg/100 g per min in controls.

The possibility that there might be a state of hyperparathyroidism was considered since parathyroid hormone reduces renal tubular phosphate reabsorption (1, 14--16) and since the phosphoruresis was accompanied by hypercalcemia in magnesium-depleted animals. MacIntyre, Boss, and Troughton (20) observed that the injection of pure parathyroid hormone produces a marked fall in magnesium and calcium excretion not accompanied by changes in creatinine excretion. They suggested that calcium and magnesium homoeostasis are interdependent and that changes in the concentration of either ion will vary the secretion of parathyroid hormone. Support for this hypothesis was recently given by Kukolj, Gitelman, and Welt (19) who found that the hypercalcemia of magnesium depletion did not obtain in the absence of the parathyroid glands even when there was replacement of the parathyroid glands with a commercial extract. The hypercalcemia was presumably dependent on an increased secretion of parathyroid hormone. In contrast, each of their magnesium-deficient groups continued to have hyperphosphaturia, i.e., this element was not corrected by parathyroidectomy.

In our experiments both the hypomagnesemic rats with intact parathyroid glands and the hypomagnesemic parathyroidectomized animals had significantly lower tubular maxima for phosphate than did their corresponding control groups. As would be expected (34, 35) the parathyroidectomized groups had higher Tm's for phosphate than the nonparathyroidectomized animals. These data suggest that although a state of relative hyperparathyroidism exists in hypomagnesemic rats which probably accounts for the associated hypercalcemia, other factors are at least partially responsible for the phosphoruresis.

REFERENCES


