

Effect of Magnesium Depletion on Responsiveness to Parathyroid Hormone in Parathyroidectomized Rats

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ABSTRACT Hypocalcemia and resistance to exogenous parathyroid hormone have been reported in several clinical states associated with magnesium deficiency. On the basis of such observations, it has been suggested that magnesium depletion per se may result in impaired responsiveness of the adenylyl cyclase-adenosine 3',5'-monophosphate (3',5'-AMP) system. To test this hypothesis, 4 wk old male parathyroidectomized rats were maintained on normal or magnesium-deficient diets for 4 wk and their responses to parathyroid hormone compared.

Serum magnesium and calcium fell progressively in the magnesium-deficient group. Despite clinical and biochemical evidence of severe magnesium deficiency in these animals, renal production and excretion of 3',5'-AMP in response to parathyroid hormone was normal both in vitro and in vivo. Additionally, administration of either dibutyl 3',5'-AMP or parathyroid extract to fasting magnesium-depleted rats produced a normal increase in serum calcium. Parathyroid hormone infusion studies demonstrated normal renal and skeletal responsiveness as measured by urinary excretion of calcium, magnesium, phosphate, and hydroxyproline. These data show that the effect of parathyroid hormone on 3',5'-AMP formation and excretion, the responsiveness of skeletal tissue to 3',5'-AMP, and the renal and skeletal system responses to parathyroid hormone are not altered by pure magnesium deficiency in the parathyroidectomized rat.

INTRODUCTION

Hypocalcemia has been reported in association with magnesium depletion in a variety of clinical states,

Dr. Hahn is recipient of U. S. Public Health Service Special Fellowship 5 PO3 CA39622-03. Dr. Chase is an Investigator, Howard Hughes Medical Institute. Dr. Avioli is a Career Research Development Awardee (7 K3 GM 22-676-05).

Received for publication 30 August 1971 and in revised form 8 November 1971.

including familial hypomagnesemia (1-3), alcoholism (4), chronic steatorrhea (5, 6), and magnesium deprivation in human volunteers (7, 8). In magnesium-depleted normal volunteers and individuals with familial hypomagnesemia, the hypocalcemia is often associated with mild elevations in serum phosphate. In all instances, serum calcium and phosphorus return to normal after treatment with magnesium supplements (1, 2, 7, 8). Recently, it has been reported that individuals with severe hypomagnesemia secondary to chronic alcoholism (4) or steatorrhea (6) are resistant to the effects of parathyroid hormone. These observations have led to the hypothesis that magnesium depletion results in decreased responsiveness of the magnesium-requiring enzyme adenylyl cyclase to parathyroid hormone with consequent decreased formation of adenosine 3',5'-monophosphate (3',5'-AMP)¹ in target tissues (4). Since this nucleotide is thought to mediate the intracellular effects of parathyroid hormone (9-11) the hypocalcemia associated with magnesium deficiency could be attributed to decreased accumulation of 3',5'-AMP in tissues normally sensitive to the hormone.

The current studies were carried out to test the effects of parathyroid hormone on the adenylyl cyclase-3',5'-AMP system in parathyroidectomized rats maintained on diets deficient in magnesium. The studies showed that pure magnesium deficiency did not impair formation of 3',5'-AMP in response to parathyroid hormone or alter the physiological effects of exogenous parathyroid hormone or 3',5'-AMP on renal and skeletal tissues.

METHODS

Diet. 5-wk old male Sprague-Dawley rats weighing approximately 120 g were parathyroidectomized by electrocautery and fed chow containing 0.6% calcium. 4 days later all animals with serum calcium less than 7.0 mg/100 ml were divided randomly into two groups and maintained on

¹Abbreviations used in this paper: 3',5'-AMP, adenosine 3',5'-monophosphate; 3',5'-GPM, guanosine 3',5'-monophosphate.

a diet deficient in magnesium (General Biochemicals, Chagrin Falls, Ohio) or an identical diet containing supplemental magnesium phosphate (12). Each diet contained a standard vitamin fortification mix and was nutritionally adequate in all respects, except for magnesium where deleted. Mineral composition of both diets as determined by direct analysis in this laboratory is shown in Table I. Demineralized water was allowed ad lib. Serum calcium and magnesium were determined initially and then after 1, 2, and 4 wk of maintenance on the test diets.

Tissue analysis. At the end of an experimental period, the animals were decapitated, and femurs, kidneys, and the heart were rapidly removed. Femurs were immediately placed in boiling distilled water for 2 min, cleaned of soft tissue and marrow and then ashed at 500°C for 18 hr. The ash was weighed and dissolved in 3 N HCl and portions were taken for determination of calcium, phosphorus, magnesium, and potassium. Kidneys and hearts were rinsed with iced normal saline, blotted with filter paper, dried at 110° C, weighed, and ashed at 500°C for 18 hr. The ash was dissolved in 3 N HCl and analyzed for magnesium.

Infusion studies. The effect of parathyroid hormone on the rate of excretion of urinary electrolytes and hydroxyproline was tested. Fasted animals were placed in a restraining apparatus and infused with a Harvard infusion pump (Harvard Apparatus Co., Inc., Millis, Mass.) at a constant rate through a No. 23 scalp vein needle inserted into a tail vein. The infusion solution contained 4% dextrose, 22 mM NaCl, and 0.1% bovine serum albumin. Using this procedure, animals can be maintained for periods of 48 hr or longer (13). Urine was collected for determination of calcium, phosphorus, magnesium, creatinine, and hydroxyproline in calibrated 100-ml tubes containing 10 ml of toluene. Urine was collected initially for 18 hr with constant infusion at a rate of 3.2 ml/hr. The infusion rate was then increased to 4.2 ml/hr and after 2 hr equilibration, specimens were collected for a total of 60–90 min at timed intervals for determination of phosphorus, 3',5'-AMP and guanosine 3',5'-monophosphate (3',5'-GMP). At the end of the control period, 10 U of purified parathyroid hormone (The Wilson Laboratories, Chicago, Ill., lot #146147) in 0.5 ml of infusing solution were injected intravenously over 2 min, and urine specimens obtained as before for an additional 60–90 min. The infusion rate was then decreased to 3.2 ml/hr and urine collected for a second 18 hr period with parathyroid hormone infused at a rate of 5 U/hr.

In vitro incubations. Slices of kidney cortex approximately 250 μ in thickness were prepared with a Stadie-Riggs microtome. Slices were incubated in a shaking incubator at 37°C for 1 hr in modified Krebs-Ringer bicarbonate buffer lacking magnesium. The buffer solution also contained theophylline 10 mM, glucose 200 mg/100 ml, and bovine plasma albumin 0.25% (w/v). The slices were then transferred to fresh buffer with or without added purified parathyroid hormone (The Wilson Laboratories) and incubated for an additional 15 min. The slices were next boiled in 1 ml of 0.05 M acetate buffer, pH 6.2, for 3 min, sonicated for 30 sec, and the residue removed by centrifugation at 10,000 *g* for 30 min. No significant loss of 3',5'-AMP or 3',5'-GMP was detectable during these extraction procedures. The clear supernatant solutions of medium and tissue extract were assayed directly for 3',5'-AMP and 3',5'-GMP.

Chemical methods and materials. Calcium and magnesium were determined by atomic absorption spectrometry (14), and phosphorus by the ascorbic acid microdetermination technique (15). Creatinine (16), total protein (17), sodium,

TABLE I
Electrolyte Content of Diets*

	Control	Low magnesium
Mg, mg	63	1.4
Ca, mg	603	592
PO ₄ , mg	578	568
Na, mEq	23	23
K, mEq	9.5	20

* Per 100 g.

and potassium (18) were determined by Technicon Auto-analyzer (Technicon Co., Inc., Tarrytown, N. Y.). Hydroxyproline was determined by the technique of Prockop and Udenfriend (19). The concentration of 3',5'-AMP and 3',5'-GMP in urine, tissue, and media was assayed according to the radioimmunoassay technique of Steiner, Parker, and Kipnis (20). Parathyroid extract (100 U/ml) was obtained from Eli Lilly and Co. (Indianapolis, Ind.), partially purified parathyroid hormone (1,000 U/mg) from The Wilson Laboratories (Chicago, Ill.), and dibutyryl 3',5'-AMP from Calbiochem (Los Angeles, Calif.). All other reagents were the highest quality available from standard suppliers.

RESULTS

Effect of magnesium depletion on calcium and magnesium in serum and tissues. Rats maintained on the magnesium-deficient diet for 10–13 days developed characteristic hyperemia of the ears and neuromuscular irritability, and by 28 days were hyperexcitable and developed convulsions if stimulated by a sudden noise. Serum calcium and magnesium in the experimental group declined steadily, becoming significantly lower than control values by 2 wk (Table II). By 4 wk, serum calcium and magnesium were 17% and 44% below control values, respectively. Serum phosphorus and total protein were not significantly different. Each group of rats gained weight throughout the test period, although the magnesium-depleted group gained less (47 g) than the controls (83 g).

Tissues from the control and magnesium-depleted animals were analyzed after 5 wk on the test diet. Results are shown in Table III. Skeletal magnesium decreased by 48% in the magnesium-depleted group, whereas skeletal calcium increased by 8% relative to control values. Similarly, magnesium concentration in the magnesium-depleted animals was decreased by 24% in renal tissue and 11% in cardiac tissue. These changes are characteristic of those reported previously for severe magnesium depletion (21–23).

Effect of parathyroid hormone on urinary 3',5'-AMP and 3',5'-GMP in magnesium-deficient rats. Infusion of 10 U of parathyroid hormone intravenously caused a rapid rise in urinary excretion of 3',5'-AMP and phosphate. The peak in urinary 3',5'-AMP preceded the

TABLE II
Effect of Magnesium Depletion on Electrolytes in Serum

	4 days after parathyroidectomy		Week 1		Week 2		Week 4	
	Control (12)	Magnesium- deficient (12)	Control (12)	Magnesium- deficient (12)	Control (12)	Magnesium- deficient (12)	Control (12)	Magnesium- deficient (10)
Ca, mg/100 ml	6.73 ±0.19	6.68 ±0.21	6.70 ±0.17	6.32 ±0.11*	6.74 ±0.22	5.84 ±0.16†	6.78 ±0.21	5.54 ±0.15‡
Mg, mg/100 ml	1.65 ±0.08	1.70 ±0.08	1.60 ±0.08	1.43 ±0.06	1.66 ±0.07	1.26 ±0.05‡	1.72 ±0.07	0.95 ±0.05‡
PO ₄ , mg/100 ml							11.7 ±0.7	12.6 ±0.8
Total protein, g/100 ml							6.1 ±0.9	6.0 ±1.0

All values given as mean ±SEM. Numbers in parenthesis indicate the number of animals.

* Significantly different from controls at $P < 0.05$.

† Significantly different from controls at $P < 0.01$.

‡ Significantly different from controls at $P < 0.001$.

maximum phosphaturic response, but 3',5'-AMP excretion then fell rapidly toward base line while phosphaturia persisted (Fig. 1), a pattern identical to that previously observed in parathyroidectomized rats (9). The basal rate of excretion of 3',5'-AMP, maximal rate of increase, total increase, and time course after parathyroid hormone were similar in each group.

In vitro production of 3',5'-AMP. The effect of parathyroid hormone on accumulation of 3',5'-AMP and 3',5'-GMP in renal tissue from magnesium-depleted rats was further tested in vitro. Slices of kidney cortex from control and magnesium-deficient rats were incubated with and without parathyroid hormone (1 U/ml) in Krebs-Ringer bicarbonate buffer lacking magnesium. The concentration of 3',5'-AMP increased from 0.40 ± 0.04 (nmoles/g wet weight) to 2.64 ± 0.20 in controls, and from 0.73 ± 0.04 to 3.32 ± 0.38 in the magnesium-deficient group (Fig. 2). The amount of 3',5'-AMP released into the medium was also similar for

each group. Parathyroid hormone did not affect the concentration of 3',5'-GMP in tissues or medium from either group. Activity of adenylyl cyclase was not assayed since this determination requires adding magnesium to the incubation medium.

Effect of dibutyryl 3',5'-AMP and parathyroid extract on serum calcium and magnesium in magnesium-deficient rats. Since magnesium deficiency did not alter the effect of parathyroid hormone on excretion of 3',5'-AMP in vivo, the possibility was tested that hypomagnesemic animals might be relatively resistant to the effects of 3',5'-AMP. After an overnight fast, dibutyryl 3',5'-AMP (100 mg/kg) was injected intraperitoneally into control and magnesium-deficient rats. Serum calcium and magnesium were determined before and 3 hr after injection. The next day, response to parathyroid extract (150 U/kg) was determined in a similar manner. Results are shown in Fig. 3.

In response to dibutyryl 3',5'-AMP, serum calcium rose 23% and 32% and serum magnesium rose 33% and 52% in control and magnesium depleted animals, respectively. A similar response was detected after injecting parathyroid extract. Since the animals had been fasted for 18 hr before each study, these responses

TABLE III
Tissue Electrolyte Concentrations

	Control (8)	Magnesium- deficient (9)
Femur (% ash weight)		
Mg	0.689 ±0.031	0.360 ±0.023*
Ca	36.9 ±0.4	38.7 ±0.6†
Na	0.551 ±0.26	0.534 ±0.019
K	0.526 ±0.033	0.244 ±0.029‡
Kidney (% dried weight)		
Mg	0.084 ±0.003	0.064 ±0.005‡
Heart (% dried weight)		
Mg	0.130 ±0.003	0.116 ±0.004‡

All values given as mean ±SEM. Numbers in parenthesis indicate the number of animals.

* Significantly different from controls at $P < 0.05$.

† Significantly different from controls at $P < 0.01$.

‡ Significantly different from controls at $P < 0.001$.

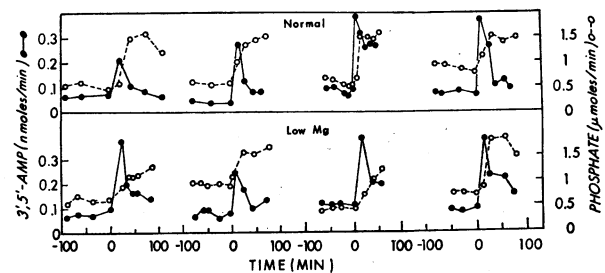


FIGURE 1 Effect of parathyroid hormone on urinary excretion of 3',5'-AMP (●) and phosphate (○) in normal and magnesium-depleted rats. Parathyroid hormone (10 U) was infused acutely through a tail vein at 0 min.

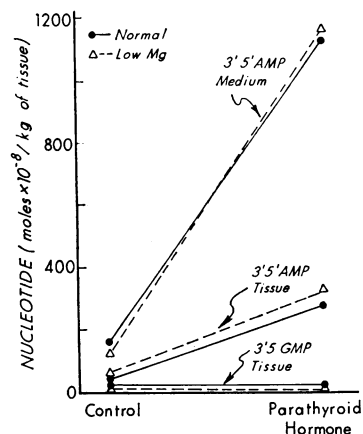


FIGURE 2 Effect of parathyroid hormone on in vitro production of cyclic AMP and cyclic GMP by renal cortex slices from normal (●) and magnesium-depleted (△) rats. Tissues were incubated for 15 min in magnesium-free bicarbonate buffer. Parathyroid hormone was employed at a concentration of 1 U/ml. Each point represents the mean of three determinations.

presumably represent release of calcium and magnesium from bone and soft tissue.

Effect of parathyroid hormone on excretion of electrolytes and hydroxyproline. Urine was collected for 18 hr before and during infusion of parathyroid hormone, 5 U/hr. Creatinine excretion was similar for each group during the base line (control rats 0.28 ± 0.02 mg/hr; magnesium-deficient rats 0.26 ± 0.02 mg/hr) and experimental (control rats 0.28 ± 0.01 mg/hr; magnesium-depleted rats 0.27 ± 0.02 mg/hr) periods. During the base line period, hydroxyproline excretion was similar for each group, although the magnesium-depleted group averaged slightly, but not significantly, lower rates of excretion (Fig. 4). In the magnesium-

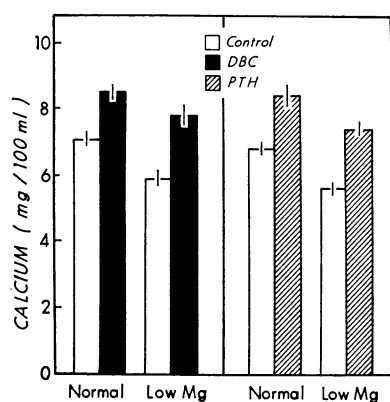


FIGURE 3 The effects of parathyroid hormone and dibutyl 3',5'-AMP (DBC) on serum calcium in normal and magnesium-depleted rats. Parathyroid hormone (150 U/kg) or DBC (100 mg/kg) were given intraperitoneally to fasting animals and serum calcium determined 3 hr later.

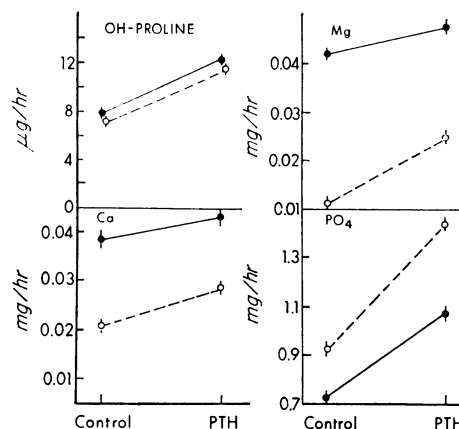


FIGURE 4 Effect of parathyroid hormone on urinary excretion of hydroxyproline (OH-proline), calcium, magnesium, and phosphate in control (●) and magnesium-depleted (○) rats. Initial urinary excretions were determined during an 18 hr overnight collection with constant infusion through a tail vein at a rate of 3.2 ml/hr with the animals in a restraining apparatus (see Methods). Parathyroid hormone (5 U/hr) was then infused during a second 18 hr overnight period. Each point represents the mean \pm SEM of eight animals.

depleted animals, urinary excretion of calcium, magnesium, and phosphorus averaged 46, 24, and 133% of control values, respectively. During infusion of parathyroid hormone, there was a brisk increase in urinary excretion of hydroxyproline, calcium, magnesium, and phosphorus in each group.

DISCUSSION

Earlier studies of the effects of magnesium depletion on calcium metabolism in the rat (24, 25) demonstrated a consistent pattern of elevated serum calcium and decreased serum phosphorus. These findings were opposite to those observed in man and other species and suggested increased secretion of parathyroid hormone. Subsequent studies showing that intact parathyroid glands were necessary for the development of hypercalcemia in magnesium-deficient rats (26, 27) substantiated this hypothesis. These findings were in accord with the observation that the rate of secretion of parathyroid hormone is increased by a low concentration of magnesium and decreased by a high concentration of this cation both in vitro (28) and in vivo (29, 30). Recently, MacManus and Heaton (31) have shown that magnesium-deficient rats maintained on a low calcium intake develop hypocalcemia and hyperphosphatemia. Thus, the seeming paradox of hypercalcemia in magnesium-depleted rats with intact parathyroid glands presumably reflects the intrinsic ability of the rat intestine to absorb an unusually high proportion of dietary calcium (32), combined with stimulation of intestinal

calcium absorption by an increased rate of secretion of parathyroid hormone.

In the current study, rats were parathyroidectomized to avoid uncertainties introduced by possible alterations in secretion of parathyroid hormone. In these parathyroidectomized animals, magnesium depletion was associated with a progressive fall in serum calcium, indicating that neither alterations in secretion of parathyroid hormone nor end-organ unresponsiveness to the hormone could be invoked as the explanation for the hypocalcemia. Also, the hypomagnesemic animals were at least as responsive as controls to the calcemic, magnesemic, and phosphaturic effects of parathyroid hormone. Normal responsiveness of the skeletal tissue to parathyroid hormone was also demonstrated by increased urinary excretion of calcium and hydroxyproline in fasted animals. These changes correlated well with the observed increases in 3',5'-AMP in response to parathyroid hormone both in vivo and in vitro. Normal responsiveness to parathyroid hormone has also been reported in patients with primary hypomagnesemia (3, 4), and in rats (26, 27) and puppies (33) maintained on diets deficient in magnesium. Since bone is the primary reservoir for magnesium, it is unlikely that the concentration of magnesium in soft tissue would fall to levels incompatible with normal activity of adenyl cyclase, even in the face of severe magnesium deficiency. This is supported by our finding that parathyroid hormone caused accumulation of 3',5'-AMP at a normal rate in cortical slices obtained from the kidneys of hypomagnesemic rats. Thus, other factors must be considered to explain the hypocalcemia associated with magnesium depletion.

Decreased secretion of parathyroid hormone is unlikely in view of previous reports (24-27) suggesting that secretion of the hormone is actually increased by these conditions and studies showing that hypomagnesemic calves develop parathyroid hyperplasia (34). Increased release of thyrocalcitonin is also unlikely since secretion of this hormone is directly rather than inversely related to the concentration of magnesium in serum (35). Since hypomagnesemic humans (1, 36) and rats (7) show decreased urinary excretion of calcium, excessive renal losses cannot explain the hypocalcemia. In the current study, the hypomagnesemic animals also showed a marked decrease in urinary excretion of calcium.

Magnesium depletion is associated with an increased concentration of calcium in soft tissues, bone, and the total carcass (21-23, 27, 37). The increased content of calcium in skeletal tissue observed in the current study is in accord with these findings. Hence, it might be speculated that an exchange of calcium for sites in the extracellular and intracellular fluid spaces formerly

occupied by magnesium contributes to the decrease in serum calcium seen in magnesium deficiency. Also, it has been suggested recently that bone from magnesium-depleted rats may retain calcium in vivo (31) and in vitro (38) due to decreased activity of both active and passive exchange processes. We are unable to support this hypothesis in the current study since basal urinary excretion of hydroxyproline was similar in control and hypomagnesemic rats, and parathyroid extract caused a marked increase in excretion of hydroxyproline in each group. It is possible, however, that smaller amounts of parathyroid extract might have elicited a differential response.

Gastrointestinal factors may also be important in the hypocalcemia of magnesium deficiency. Absorption of calcium is not impaired in hypomagnesemic states (39-41); however, absorption of phosphorus may be increased in the intestine of magnesium-deficient animals (40). The resulting increase in phosphate absorption would tend to elevate serum phosphate with a consequent shift in the calcium-phosphate equilibrium toward increased deposition of calcium in soft tissue and bone. It is of interest that mild elevations of serum phosphorus have frequently been noted with magnesium depletion in man (1-6) and various animal species (22-26). Also, increased urinary excretion of phosphorus in conjunction with normal serum phosphate as reported here and in previous studies (25, 26, 31) is consistent with an increased size of the exchangeable pool of phosphate.

The current studies demonstrate that hypomagnesemic rats respond normally to parathyroid hormone. The data indicate that severe magnesium deficiency in this species is not associated with decreased responsiveness of the adenyl cyclase-3',5'-AMP system to parathyroid hormone. Development of hypocalcemia in hypomagnesemic states might be attributed to altered equilibrium between intra- and extravascular calcium and magnesium as well as increased gastrointestinal absorption of phosphorus. Further studies will be required to test this hypothesis. In alcoholics (4) and individuals with chronic steatorrhea (6) who manifest resistance to parathyroid hormone, factors other than pure magnesium depletion may be operative.

ACKNOWLEDGMENTS

This work was supported in part by National Institutes of Health Grants RR 00036, AM 11674, and National Institutes of Health Endocrine Training Program AM 05027.

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