PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 148, 1038–1043 (1975)

Effects of Magnesium Deficiency and Thyroparathyroidectomy on Calcium Active Transport by Rat Duodenum[1] (38684)

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(Introduced by Franklin H. Epstein)

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While man and many other animals usually develop hypocalcemia in response to Mg deficiency (1–9), the rat becomes hypercalcemic (10–20). Because this hypercalcemic response seems to be dependent upon the availability of Ca in the diet (21), increased absorption of Ca by the intestine may be involved. Since signs consistent with secondary hyperparathyroidism, such as hypophosphatemia (14, 15, 17), hyperphosphaturia (11, 13, 15, 17), and hypocalciuria (11, 14, 15) are also observed in Mg deficient animals, increased secretion of parathyroid hormone (PTH) (presumably in response to hypomagnesemia) has been suggested as the factor responsible for these changes (15, 18) as well as for the reported increases in intestinal Ca absorption (16, 19). There are, however, conflicting reports in the literature concerning the effects of both Mg deficiency (12, 16, 17, 19, 20) and PTH (19, 22–26) on Ca absorption by the rat intestine.

In the present study, we examined the effects of Mg deficiency (produced by dietary Mg deprivation) on duodenal Ca active transport in sham-operated or thyroparathyroidectomized (TPTX) rats. The results indicate that while moderate Mg deficiency does increase active Ca absorption, more severe deficiency may depress it. Furthermore, thyroparathyroidectomy (TPTX) not only abolished the transport response to Mg depletion, but also reduced duodenal Ca absorption in control animals fed a diet with normal mineral content.

Materials and Methods. Animal preparation. Male Sherman rats (Camm Research Institute, Inc., Wayne, NJ) were obtained as weanlings. To control the vitamin D status, these weanlings were initially raised in the dark for 6 wk on a vitamin D-free diet containing 0.8% Ca, 0.24% Mg and 0.4% *P* (test diet No. 69280, General Biochemicals Div., Mogul Corp., Chagrin Falls, OH). The animals were then randomized by weight into five experimental groups (mean wt for all groups = 238 g). Two groups were continued on the Mg-containing, vitamin D-free control diet described above, while the remaining three groups were fed a Mg-free diet otherwise identical to the control diet (test diet No. 72359, General Biochemicals Div., Mogul Corp., Chagrin Falls, OH). On the day of randomization and every third day until sacrifice, each animal received 2.84 nmoles (45 IU) of vitamin D_3 in 0.3 ml of propylene glycol: 95% ethanol, 4:1 (vol/vol) by gastric tube (last dose 24 hr before sacrifice). The animals were pair-fed by allowing rats on the control diet the same amount of food consumed on the preceding day by animals on the Mg-free diet. This pair feeding procedure resulted in similar weights at sacrifice with group means ranging from 260 to 300 g. Seven days after initiation of the vitamin D-repletion and pair-feeding regimen, animals underwent either surgical TPTX or sham surgery under light ether anesthesia. Postoperatively, the animals were returned to their previous diets. Except for a sham-operated group main-

[1] This work was supported by grants AM-13696 and AM-05114 from the National Institutes of Health of the U.S. Public Health Service.

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tained on the Mg-free diet for a total of 19 days (12 days postoperatively), all other animals were sacrificed after having been pair-fed a particular test diet for 10 days (third postoperative day). TPTX was considered to be complete if plasma Ca was 1.75 m*M* or less at sacrifice. Data from animals that did not meet this criterion for TPTX were excluded.

Ca transport studies. Animals were fasted overnight and sacrificed by cerebral concussion followed by exsanguination. The experimental technique for transport studies was identical to that which we previously described (27, 28) (modified Ussing-type apparatus); this method allows for the elimination of electrochemical gradients. The incubation medium was a bicarbonate-buffered Krebs-Ringer solution gassed with 95% O_2–5% CO_2, containing 0.5 m*M* Ca, no P and 11 m*M* D-glucose (28). The transmural potential difference (PD) was nulled using the method of correcting for fluid resistance described by Field, Fromm and McColl (29). All electrical parameters reported are for time periods when transmural Ca fluxes were at steady-state. Since net Ca fluxes were measured across paired pieces of proximal duodenum from the same animal, paired "t" tests were used to evaluate differences between mucosal to serosal fluxes (J_{MS}) and serosal to mucosal fluxes (J_{SM}) within each group. One way analyses of variance were used to determine whether or not differences existed between groups for J_{MS}, J_{SM}, electrical parameters and plasma Ca, Mg, and P. The probability levels of differences between the means were then evaluated by constructing tables of "least significant difference" as described by Snedecor and Cochran (30).

Plasma Ca, Mg, and P determinations. At the time of sacrifice blood was collected in heparinized tubes. Ca and Mg concentrations in plasma were measured by atomic absorption spectroscopy (Perkin-Elmer Model 290B). Phosphorus was determined according to the method of Fiske and SubbaRow (31).

Results. Animal growth on the different diets. During the initial 6 wk when all animals were fed the vitamin D-deficient diet, the mean weight gain per day was 3.5 g (range 3.1–3.7 g). When paired-feeding and vitamin D-repletion were initiated, the growth rate was 3.8 g per day during the ensuing 10 days (based on weights of surviving animals), and there were no significant differences between the Mg-fed and Mg-depleted groups. The sham-operated animals maintained on the Mg-free diet for an additional 9 days continued to grow at a rate similar to that of their 10-day counterparts (4.0 g per day). Since paired-feeding and vitamin D-repletion were initiated simultaneously, it is impossible to assess the effect of either condition alone on the growth rate.

Ca transport studies. Duodenal active Ca absorption in sham-operated animals was increased by feeding the Mg-free diet for 10 days (Table I). However, both the sham animals fed the Mg-free diet for 19 days and TPTX animals fed either the control or Mg-free diets had even lower levels of Ca absorption than did the sham group fed the control diet (Table I). The decreased active Ca absorption in these three groups resulted from both decreases in J_{MS} and increases in J_{SM} (Table I). Both TPTX and prolonged feeding of the Mg-free diet increased Ca J_{SM} and electrical conductance (G) (Table I). The short-circuit current (SCC) was also increased by TPTX in both the control and Mg-deficient animals (Table I). Even though the conductance was higher across intestine from TPTX animals, the open-circuited PD in these groups was greater than the PD in sham-operated animals because of the larger SCC in the TPTX groups (PD = SCC/G, Table I).

Plasma Ca, Mg and P. After 7–10 days on the Mg-free diet animals exhibited such signs of Mg deficiency as hyperemic ears, occasional skin lesions, and a tendency towards hyperexcitability. The existence of Mg deficiency was confirmed by a progressive decrease in plasma Mg levels with increasing time on the Mg-free diet in the sham-operated groups (Table II). The plasma Mg content was also lower in the TPTX group fed the control diet than in the comparable sham group, and still lower plasma Mg levels were observed in the TPTX group fed the Mg-free diet (Table

TABLE I. EFFECTS OF DIETARY MAGNESIUM LEVELS AND THYROPARATHYROIDECTOMY ON DUODENAL CALCIUM TRANSPORT AND ELECTRICAL PARAMETERS.

Condition[a]	n	Calcium fluxes, nmoles·cm^{-2}·hr^{-1} J_{MS}	J_{SM}	J_{Net}	SCC μA·cm^{-2}	G mmhos·cm^{-2}
Sham, Control diet	7	15.8 ± 2.2[b]	3.7 ± 0.2	12.1 ± 2.1[c]	33.0 ± 1.1	7.9 ± 0.6
Sham, Mg-free diet (10 days)	7	21.7 ± 1.2[d]	3.7 ± 0.4	18.0 ± 1.1[e, f, g]	44.7 ± 2.9	8.0 ± 0.4
Sham, Prolonged Mg-free diet (19 days)	8	11.6 ± 0.8	4.7 ± 0.3[h]	6.9 ± 0.6	44.1 ± 3.4	10.1 ± 0.8[h]
TPTX, Control diet	5	11.8 ± 1.3	5.1 ± 0.4[h]	6.7 ± 1.0	55.4 ± 6.3[i]	9.9 ± 0.9[h]
TPTX, Mg-free diet (10 days)	5	11.8 ± 1.5	5.3 ± 0.3[h]	6.5 ± 1.6	65.7 ± 9.7[i]	9.8 ± 0.9[h]

[a] See Methods for detailed descriptions of diets, vitamin D-supplementation, confirmation of TPTX, and Ca flux measurements. All values ±SEM. J_{MS} is Ca flux from the mucosal to serosal surface of intestine, J_{SM} is flux in the opposite direction and $J_{Net} = J_{MS} - J_{SM}$.

[b] Sham, control diet J_{MS} > Sham, control diet J_{SM}; $P < 0.001$.

[c] Sham, control diet J_{Net} > TPTX, control diet J_{Net}; $P < 0.05$.

[d] Sham, Mg-free diet J_{MS} > Sham, Mg-free diet J_{SM}; $P < 0.001$.

[e] Sham, Mg-free diet J_{Net} > Sham, control diet J_{Net}; $P < 0.05$.

[f] Sham, Mg-free diet J_{Net} > Sham, prolonged Mg-free diet J_{Net}; $P < 0.001$.

[g] Sham, Mg-free diet J_{Net} > TPTX, Mg-free diet J_{Net}; $P < 0.001$.

[h] J_{SM} and conductance (G) from Sham, prolonged Mg-free diet and both TPTX groups > J_{SM} and conductances from Sham groups on diet 10 days; $P < 0.05$ or greater.

[i] SCC in both TPTX groups > SCC in all Sham groups; $P < 0.01$ or greater.

II). Plasma Ca levels were much lower in both TPTX groups and as expected, plasma P levels were lower in both sham groups fed the Mg-free diet than in the sham group fed the control diet (Table II).

Discussion. The degree of hypomagnesemia which resulted from dietary Mg deprivation in the current study is similar to that previously reported for equivalent times on diet (11, 20, 32). Since hypercalcemia occurs in Mg deficient rats only when there is an adequate source of dietary Ca (21), the 16–18 hr of fasting prior to sacrifice may account for our failure to observe this phenomenon (Table II). The lower plasma P levels in the Mg-deficient, sham-operated groups relative to control diet animals (Table II), are consistent with the findings of Chutkow (14). One cause of this hypophosphatemia could be increased PTH secretion.

The results of the present investigation confirm and extend the observations of Kessner and Epstein (16) and Morehead and Kessner (19) that feeding an essentially Mg-free diet to intact rats for 10 days produces increased duodenal Ca absorption. Furthermore, the fact that this dietary regimen did not increase Ca absorption when animals were either surgically parathyroidectomized (PTX) (19) or TPTX (present study) suggests that PTH is somehow involved in increasing Ca absorption in response to Mg deprivation. In addition, our results provide a clearcut demonstration of an effect of TPTX *per se* (72 hr postoperatively) on active Ca absorption, since in both TPTX groups net absorption was decreased to levels even lower than those observed in the sham-operated, control diet group (Table I). Since thyroid hormone (33) and calcitonin (34) have both been reported to cause decreases in Ca transport, it is unlikely that thyroidectomy can account for the changes in active Ca absorption observed in the present study 72 hr after TPTX (Table I).

Of special note is the fact that the present experiments, conducted under short-circuited conditions provide the first demonstration that the effects of Mg deficiency and acute TPTX on Ca absorption involve alterations in the Ca active transport process *per se*. Open-circuited everted gut sac preparations

TABLE II. CHANGES IN PLASMA CALCIUM, MAGNESIUM AND PHOSPHORUS LEVELS FOLLOWNG DIETARY MAGNESIUM RESTRICTION AND THYROPARATHYROIDECTOMY.

Condition[a]	Plasma concentration, mM[a]					
	n	Calcium	*n*	Magnesium	*n*	Phosphorus
Sham, Control diet	12	2.51 ± 0.03	12	0.85 ± 0.01[c, d]	11	3.71 ± 0.08[g]
Sham, Mg-free diet (10 days)	14	2.58 ± 0.03	14	0.50 ± 0.01	13	2.69 ± 0.05
Sham, Prolonged Mg-free diet (19 days)	8	2.53 ± 0.03	8	0.33 ± 0.02	8	2.58 ± 0.10
TPTX, Control diet	11	1.26 ± 0.03[b]	11	0.73 ± 0.03[e]	11	5.35 ± 0.24[h]
TPTX, Mg-free diet (10 days)	11	1.20 ± 0.02[b]	11	0.53 ± 0.02[f]	11	5.01 ± 0.19[h]

[a] See Methods for detailed descriptions of diets, vitamin D-supplementation, confirmation of TPTX and plasma Ca, Mg and P determinations. All values are ±SEM.

[b] Plasma Ca from all Sham groups > plasma Ca, TPTX groups, $P < 0.001$.

[c] Sham, control diet plasma Mg > Sham, Mg-free diet plasma Mg, $P < 0.001$.

[d] Sham, control diet plasma Mg > TPTX, control diet plasma Mg, $P < 0.001$.

[e] TPTX, control diet plasma Mg > TPTX, Mg-free diet plasma Mg, $P < 0.001$.

[f] TPTX, Mg-free diet plasma Mg > Sham, prolonged Mg-free diet, $P < 0.001$.

[g] Sham, control diet plasma P > plasma P in both Sham, Mg-free diet groups, $P < 0.001$.

[h] Plasma P from both TPTX groups > plasma P, all Sham groups, $P < 0.001$.

have been employed in most in vitro studies of the effects of Mg deficiency (16, 17, 19, 20) and PTX (22–24, 26) on intestinal Ca absorption. The distinction between active transport measured under short-circuited conditions and net absorption assessed by open-circuited techniques may be important since in the TPTX groups (present study) for example, both the intestinal PD and conductance were greater than in comparable sham-operated control animals (Table I). Theoretically, the combined effects of increases in PD and tissue conductance will decrease the serosal compartment ^{45}Ca/mucosal compartment ^{45}Ca ratios that would be observed in everted gut sacs. Therefore, an observed decrease in Ca absorption in an open-circuited preparation could result from changes in diffusional movements and need not necessarily reflect changes in active transport.

Two other groups of investigators failed to observe changes in Ca absorption after feeding Mg deficient diets for the same (20) or more prolonged periods (17, 20). However, in both of these studies the Mg content of the control diets was only 0.04–0.053% (17, 20), while our control diet and that used by Morehead and Kessner (19) contained 0.24% and 0.11% Mg, respectively. The observation of signs of secondary hyperparathyroidism in animals fed a diet with a Mg content of 0.04% led Martindale and Heaton to the conclusion that this was a suboptimal level for a control diet (32). Therefore, it appears reasonable to suggest that Lifshitz and his coworkers (17) and Krawitt (20) failed to find enhanced Ca absorption because of the effects of the low levels of Mg in the control diets. If duodenal Ca absorption was already enhanced as a result of the low Mg content of the control diet in the studies mentioned above (17, 20), it is conceivable that further increases in absorption in response to a diet with an even lower Mg content either might not occur or might be undectable.

While the effects of moderate Mg deficiency and of TPTX on active intestinal Ca absorption observed in the present study may be indicative of direct effects of PTH on the intestine, it seems more likely that they are the result of alterations in vitamin D metabolsim. Recent evidence indicates

that 1,25-dihydroxyvitamin D_3 (1,25-$(OH)_2D_3$), produced in the kidney from 25-hydroxyvitamin D_3 (25-OH D_3), is the major metabolite of vitamin D which acts on intestine to increase Ca absorption (35). In experiments similar to those presented in this report, Garabedian and her coworkers (36) demonstrated that within 48 hours after TPTX of rats on a restricted dietary Ca intake, 1,25-$(OH)_2D_3$ disappears from the blood and intestinal mucosa and under these circumstances injection of PTH restores 1,25-$(OH)_2D_3$ levels to normal. These workers did not investigate the effects of TPTX on Ca absorption however. In accordance with such a PTH effect on 1,25-$(OH)_2D_3$ synthesis, acute removal of the parathyroids could account for diminished intestinal Ca absorption observed in the present studies which were conducted 72 hours after surgery in animals with limited vitamin D stores.

Finally, one must consider the factors that might play a role in the decreased Ca active transport observed in more severely hypomagnesemic sham-operated animals (due to more prolonged dietary Mg deprivation). It has recently been suggested that the severe Mg deficient state in man may be associated with impairment in PTH secretion (4, 5); however, there is also evidence that altered end-organ responsiveness to PTH may be involved (2, 3). Although the ability of rat kidney adenylate cyclase to respond to PTH is apparently intact in the Mg deficient state (37), other data have suggested that the end-organ responsiveness of bone and kidney to this hormone is somehow impaired (38). It is difficult to document the presence of glandular malfunction in the rat because unlike human PTH, the rat hormone is not yet readily measureable by immunoassay. The current demonstration of a TPTX-like depression of Ca absorption by prolonged Mg deficiency could therefore be due to a failure of PTH secretion, failure of the renal 25-OH D_3-1-hydroxylase system to respond to PTH, or inability of the intestine to respond to either "adequate" amounts of 1,25-$(OH)_2D_3$ or possibly to PTH itself as a result of the severely Mg-depleted state. The resolution of many of the questions raised by this and earlier studies of experimental Mg deficiency will require a direct means of measuring PTH secretion in the rat as well as investigation of the effects of Mg deficiency on the metabolism of vitamin D.

Summary. Mg deficiency was produced in rats by feeding a Mg-free diet. Ten days of dietary Mg depletion led to an increase in active duodenal Ca absorption in sham-operated animals, but this increase was abolished by thyroparathyroidectomy (TPTX). In addition, TPTX reduced Ca absorption in control animals fed a Mg-containing diet. More prolonged Mg deficiency was produced by feeding sham-operated animals the Mg-free diet for 19 days. This condition resulted in more marked hypomagnesemia and a depression of Ca transport rates to the level observed in the TPTX groups. These results are consistent with the concept that adaptation of duodenal Ca transport in response to Mg deficiency occurs through an increase in parathyroid hormone (PTH) secretion; however, direct blood PTH measurements will be required to prove this point.

We would like to thank Gail Millar Fishbein for her expert technical assistance.

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Received July 22, 1974. P.S.E.B.M. 1975, Vol. 148.