Amino acid, water and electrolyte transport in the small intestine of magnesium depleted rats

By *C. H. R. Thirumalai*

Department of Physiology, University of the West Indies, Jamaica, West Indies

Zusammenfassung

Die Wirkung einer magnesiumarmen Diät auf den Aminosäure-, Wasser- und Elektrolyt-Transport im Dünndarm wurde mit einer Einmaldurchlauf-Perfusionstechnik an der Ratte unter *in-vivo*-Bedingungen untersucht.

Magnesium-Verarmung während 28 Tagen beeinträchtigte die Alanin- oder Lysin-Absorption der untersuchten Dünndarmstellen nicht; der Transport von Natrium-Ionen, Chlorid-Ionen und Wasser aber wurde sehr stark gehemmt. Diese Hemmung zeigte sich vor allem bei 50 mM Alanin/Kochsalzlösungen, und zwar an allen untersuchten Dünndarmarialen.

Summary

The effect of dietary magnesium depletion on amino acid, water and electrolyte transport from the small intestine *in vivo* has been studied by a single pass perfusion technique in the rat.

Twenty eight days of magnesium depletion did not affect either alanine or lysine absorption by any part of the small intestine, but did severely inhibit sodium ion, chloride ion and water transport especially from 50 mM alanine/saline solutions in all regions of the small intestine which were studied.

Résumé

L'effet de l'épuisement de magnésium par diète sur le transport *in vivo* des acides aminés, de l'eau et des électrolytes dans l'intestin grêle a été étudié par une technique de perfusion.

L'épuisement de magnésium pendant 28 jours ne changeait pas l'absorption de l'alanine ou de lysine dans aucune partie de l'intestin grêle, mais on observait une forte inhibition du transport de sodium, de chlorure et de l'eau dans toutes les parties étudiées de l'intestin grêle, surtout en employant des solutions de 50 mM alanine/salin.

Introduction

Dietary magnesium depletion in animals causes changes in the metabolism of calcium [9, 10], potassium [4, 11] and phosphate [5] which have been attributed to interference with the sodium-potassium pump [24]. A generalized aminoaciduria is also seen in these animals which is associated with increased activity of the renal Na^+-K^+ activated ATPase, and it has been suggested that this is due to a disturbance of energy metabolism in the renal tubular cell [12].

Disturbances of amino acid reabsorption in the kidney are often accompanied by similar transport defects in the small intestine [14, 15, 26]. It is possible, therefore, that a similar defect may exist in the small intestine of rats rendered magnesium deplete by dietary means.

Extensive *in vivo* and *in vitro* studies have demonstrated the relationship between the intestinal absorption of certain non-electrolytes (glucose and amino acids) and sodium ions. Thus, absorption of the two amino acids alanine and lysine, water, sodium ions and chloride ions in normal and magnesium depleted rats has been determined in this study and the results discussed.

Materials and methods

The net absorption rates of the amino acids L-lysine and L-alanine, sodium ions, chloride ions and water from the small intestine were studied under *in vivo* conditions in normal and magnesium depleted rats.

The net absorption rates were estimated by disappearance from the intestinal lumen using a single pass perfusion technique [7, 8, 21, 22, 23] in three regions of the small intestine, namely (a) the proximal jejunum; (b) the mid small intestine and (c) the distal ileum.

Five hundred and twenty male Wistar rats each weighing between 90 and 120 grammes were divided into two groups I and II. Group I was fed *ad libitum* on a low magnesium test diet (Nutritional Biochemicals Inc., Cleveland, Ohio, USA Cat. No. 902205) for a period of twenty eight days and was provided with distilled water. Group II was fed *ad libitum* with a standard pelleted complete feed (Purina Chow No. 5001 Ralston Purina Co. Ltd., Checkerboard Square, St. Louis, Missouri, 63188, USA) and was provided with tap water. The constituents of these diets are given in tables 1 and 2.

Perfusion solutions

Solutions of amino acids, isotonic with plasma (300 mM) were prepared and then diluted to the required concentration (10 mM or 50 mM) with isotonic saline (150 mM). Polyethylene glycol molecular weight 4000 (PEG) was added to produce a concentration of 3 g per litre. In addition, 1,2-^{14}C labelled PEG (NEN Boston, Massachusetts, USA) was added to give a concentration of 1.0 μCi per litre and tritiated amino acid to give a concentration of 10.0 μCi per litre. The pH of the solutions were adjusted to 7.0 with M^{-1} sodium hydroxide or M^{-1} hydrochloric acid as necessary. The osmolality of the solutions was checked by depression of the freezing point (Advanced Osmometer — Advanced Instruments Ltd. Newton Highlands Massa-

Table 1: Composition of normal diet (Lab Chow 5001) g/kg of diet

Protein	234.0	Copper	0.018
Arginine	13.8	Cobalt	0.0004
Cystine	3.2	Iodine	0.0017
Glycine	12.0	Fat	45.0
Histidine	6.0	Fibre	52.0
Isoleucine	12.0	Total dry nutrient	750
Leucine	16.0	Non Fat Energy	4.25 Mcal
Lysine	14.5	Ash	70.0
Methionine	4.3	Moisture	10 %
Phenylalanine	10.3	Vitamins:	
Threonine	9.4	Carotene	0.0065
Valine	12.4	Thiamine	0.0177
Calcium	12.0	Riboflavin	0.008
Phosphorous	8.6	Niacin	0.095
Potassium	10.1	Pantothenic acid	0.024
Magnesium	2.1	Choline	2.25
Sodium	4.4	Folic acid	0.0059
Chlorine	5.0	Pyridoxine	0.0038
Fluorine	0.035	Biotin	0.00007
Iron	0.198	B_{12}	0.00000559
Zinc	0.058	Vitamin A	15,000 IU
Manganese	0.051	Vitamin D	5,300 IU
		α Tocopherol	13.6 IU

Table 2: Composition of low magnesium diet as supplied by manufacturer g/kg of diet

Casein	250.0
Vegetable oil	50.0
Gelatin	50.0
Dimethionine	0.62
Dextrose	589.4
$CaCO_3$	27.63
$CaHPO_4$	6.93
$CuSO_4$	0.028
Ferric citrate	2.58
KI	0.076
NaCl	15.46
$ZnCO_3$	0.023
K_2HPO_4	6.92
$MnSO_4$	0.366
Magnesium content	0.049
Plus full vitamin fortification	

chusetts, USA) and was always found to be in the range 250 to 300 m Osmoles/kg.

The animals were fasted overnight and then anaesthetised with Nembutal (40 mg/kg) for perfusion of the small intestine. Perfusion of twenty centimetre segments of the small intestine was carried out at 0.2 ml per minute (pump rate). One hour was allowed for equilibration after which three twenty minute aliquots of the perfusion effluent were collected for chemical analysis.

Analytical methods

Sodium ion concentrations were estimated using a Technicon 6/60 autoanalyser and chloride concentrations by coulometric titration (Evans Electroselenium, Halstead, Essex, U.K.). ^{14}C and ^{3}H were estimated by liquid scintillation counting using a 1.0 ml sample in 15.0 ml of scintillation Cocktail (750.0 ml toluene, 250.0 ml Triton −X100 containing 4.0 g PPO per litre) and counted in a Beckman LS 150 automatic scintillation counter.

An estimation of the recovery of PEG was made by comparing the known delivery of PEG with that collected in the perfusion effluent. A mean recovery of 97.57 % ±9.23 % was obtained.

Calculations

Absorption rates were calculated using standard formulae [20].

Statistical methods

Wilcoxon's paired and unpaired tests were used to assess the statistical significance of the differences between the means. Value for p of less than 0,05 were taken as significant [29, 30, 31].

Results

Compared with the normal controls, animals on the magnesium deficient diet grew more slowly and eventually stopped growing (Fig. 1). Clinically, the magnesium depleted animals demonstrated the well known signs of erythema in the ears, foot pads, tail and scrotum during days five to fourteen, accompanied by increasing irritability and mortality.

Amino acid transport

The mean net absorption rates for both alanine and lysine

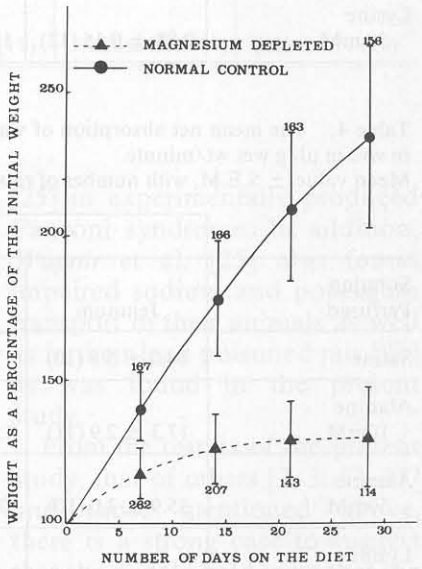

Figure 1: Weight curves for normal and magnesium depleted rats. The mean weights ± S.D. and the number of animals are shown.

increased with increasing concentration in both the normal and magnesium depleted rats. Alanine absorption rates were always greater than those for lysine. There was no statistically significant difference between the two groups of rats in relation to absorption rates for either of the two amino acids. However, alanine was absorbed faster by the mid gut than by either the jejunum or ileum ($P<0.05$). These results have been summarised in table 3.

Water transport

Mean net water absorption increased with increasing concentration of amino acid. Lysine at high concentrations (50 mM) however, tended to inhibit water absorption; but this tendency did not reach statistical significance. Magnesium depletion did not appear to affect water transport by any region of the small intestine except when 50 mM alanine was perfused; in which case, a significant reduction in water absorption rate was observed in all three regions of the small intestine ($P<0.01$). These results have been summarised in table 4.

Sodium transport

Mean net sodium absorption rates increased with increasing concentration of amino acids in normal rats. There was no statistically significant difference between normal rats and magnesium depleted rats with respect to sodium transport except when 50 mM alanine was perfused. In this case, magnesium depletion severely inhibited sodium absorption ($P<0.01$) in all three regions of the small intestine studied. These results have been summarised in table 5.

Chloride transport

Handling of chloride ion by the small intestine was very similar to that of sodium, except that the ileum absorbed greater

Table 3: The mean net transport of amino acid from mixtures with saline from perfused loops of rat small intestine *in vivo* in μmol/g wet wt/minute.
Mean value ± S.E.M. with number of rats in parentheses

	Normal rats			Magnesium deficient rats		
Solution Perfused	Jejunum	Mid Gut	Ileum	Jejunum	Mid Gut	Ileum
Alanine 10 mM	0.63 ± 0.05 (11)	0.68 ± 0.04 (12)	0.63 ± 0.05 (12)	0.65 ± 0.03 (12)	0.79 ± 0.07 (12)	0.62 ± 0.05 (12)
Alanine 50 mM	2.94 ± 0.32 (12)	3.21 ± 0.32 (10)	2.59 ± 0.25 (10)	2.34 ± 0.23 (10)	2.47 ± 0.28 (9)	2.20 ± 0.34 (10)
Lysine 10 mM	0.25 ± 0.03 (12)	0.27 ± 0.04 (12)	0.30 ± 0.03 (11)	0.24 ± 0.03 (10)	0.23 ± 0.04 (10)	0.25 ± 0.03 (10)
Lysine 50 mM	0.88 ± 0.15 (12)	1.07 ± 0.18 (12)	1.51 ± 0.29 (11)	0.94 ± 0.15 (11)	1.14 ± 0.20 (10)	1.17 ± 0.16 (11)

Table 4: The mean net absorption of water from saline and amino acid/saline mixtures from perfused loops of rat small intestine *in vivo* in μl/g wet wt/minute.
Mean value ± S.E.M. with number of rats in parentheses.

	Normal rats			Magnesium deficient rats		
Solution Perfused	Jejunum	Mid Gut	Ileum	Jejunum	Mid Gut	Ileum
Saline	14.0 ± 5.8 (10)	7.05 ± 4.8 (10)	10.0 ± 5.5 (10)	17.07 ± 7.0 (11)	7.9 ± 6.6 (10)	13.0 ± 5.9 (10)
Alanine 10mM	17.3 ± 2.9 (11)	15.71 ± 3.8 (12)	15.5 ± 3.9 (12)	20.7 ± 6.1 (12)	21.6 ± 7.3 (12)	18.5 ± 4.3 (12)
Alanine 50mM	35.9 ± 3.0 (10)	33.4 ± 3.8 (10)	32.1 ± 2.7 (10)	14.5 ± 5.7 (10)	5.1 ± 6.6 (9)	19.2 ± 7.5 (10)
Lysine 10mM	23.0 ± 3.4 (12)	21.2 ± 1.3 (12)	23.4 ± 2.4 (11)	22.9 ± 3.2 (10)	14.1 ± 3.0 (10)	21.0 ± 3.0 (10)
Lysine 50mM	11.7 ± 4.8 (12)	13.2 ± 6.7 (12)	17.4 ± 8.0 (11)	9.8 ± 4.4 (11)	13.3 ± 6.9 (11)	11.8 ± 4.5 (11)

Table 5: The mean net absorption of sodium from saline and amino acid/saline mixtures from perfused loops of rat small intestine *in vivo* in μmol/g wet wt/minute.
Mean value ± S.E.M. with number of rats in parentheses.
Negative value indicates net secretion of sodium into the gut lumen.

	Normal rats			Magnesium deficient rats		
Solution Perfused	Jejunum	Mid Gut	Ileum	Jejunum	Mid Gut	Ileum
Saline	2.05 ± 0.83 (10)	1.09 ± 0.80 (10)	1.94 ± 0.87 (10)	2.47 ± 1.21 (11)	0.42 ± 1.22 (10)	0.59 ± 1.31 (10)
Alanine 10mM	2.60 ± 0.55 (11)	2.28 ± 0.69 (12)	2.98 ± 0.83 (12)	2.86 ± 1.13 (12)	3.42 ± 1.31 (12)	2.49 ± 1.0 (12)
Alanine 50mM	4.73 ± 0.45 (10)	3.67 ± 1.02 (10)	4.61 ± 1.03 (10)	0.76 ± 1.0 (10)	− 1.12 ± 1.13 (9)	0.00 ± 0.94 (9)
Lysine 10mM	2.81 ± 0.43 (12)	2.42 ± 0.47 (12)	2.90 ± 0.67 (11)	2.80 ± 0.70 (10)	1.32 ± 0.52 (10)	2.36 ± 0.58 (9)
Lysine 50mM	2.84 ± 0.78 (12)	3.20 ± 1.03 (12)	3.81 ± 1.13 (11)	2.48 ± 0.84 (11)	2.83 ± 1.10 (11)	2.95 ± 0.75 (11)

Table 6: The mean net absorption of chloride from saline and amino acid/saline mixtures from perfused loops of rat small intestine *in vivo* μmol/g wet wt/minute.
Mean values ± S.E.M. with number of rats in patentheses.
Negative value indicates net secretion of chloride into the gut lumen.

	Normal rats			Magnesium deficient rats		
Solution Perfused	Jejunum	Mid Gut	Ileum	Jejunum	Mid Gut	Ileum
Saline	2.28 ± 1.10 (10)	0.99 ± 0.99 (10)	3.08 ± 1.10 (10)	1.44 ± 1.40 (11)	0.03 ± 1.40 (10)	2.53 ± 1.41 (9)
Alanine 10mM	3.58 ± 0.68 (11)	2.43 ± 0.63 (12)	4.64 ± 1.02 (12)	3.46 ± 1.32 (12)	3.05 ± 1.87 (12)	4.39 ± 0.93 (12)
Alanine 50mM	5.41 ± 0.75 (10)	4.20 ± 0.72 (10)	5.77 ± 0.56 (9)	2.16 ± 1.01 (10)	− 0.22 ± 1.11 (9)	3.35 ± 1.15 (10)
Lysine 10mM	2.89 ± 0.47 (12)	2.88 ± 0.60 (12)	5.35 ± 0.84 (11)	3.34 ± 0.89 (10)	1.55 ± 0.81 (10)	4.56 ± 0.35 (8)
Lysine 50mM	3.87 ± 0.70 (12)	4.35 ± 1.08 (12)	6.61 ± 1.28 (11)	4.46 ± 0.83 (11)	4.26 ± 1.19 (11)	5.89 ± 0.77 (10)

quantities of chloride ion in all situations, than did any other region of the small intestine. In a similar fashion to sodium transport, magnesium depletion greatly reduced mean chloride absorption rates in the presence of 50 mM alanine ($P < 0.01$). These results have been summarised in table 6.

Discussion

This study has demonstrated no effect of magnesium depletion on the absorption of alanine and lysine by the small intestine. This is in contrast to the observations in the kidney of the same animals [23].

The lack of an effect of magnesium depletion is of interest since in a number of similar situations, absorption of at least some amino acids is affected: lead poisoning [26]; protein energy malnutrition [1, 6, 27].

The finding that amino acid absorption is not affected by magnesium depletion is similar to the observations of *Rosenberg* and *Segal* [19] and *Wapnir* et al. [25] in experimentally produced Fanconi syndrome. In addition, *Wapnir* et al. [25] also found impaired sodium and potassium transport in their animals as well as in their lead poisoned rats [26] as was found in the present study.

From the results of the present study, that of others [2, 3, 13, 15] and those mentioned above, there is a strong case to suggest that the widely held view that the proximal tubule of the kidney and the small intestine, especially its proximal section,

behave in a similar fashion towards the absorption of amino acids and sugars, is no longer tenable. However, the question arises as to why this should be so, and why is there no consistent pattern in the effects of the different syndromes on amino acid and electrolyte absorption in either the small intestine or the kidney.

It is likely that the inhibition of electrolyte absorption in the small intestine is a result of the reduced Mg^{2+} dependent $Na^+ - K^+$ activated ATPase level, which is known to occur in magnesium depletion [28]. The fact that amino acid absorption from the small intestine was not impaired may be due to the observation that organic solute absorption by the small intestine is not totally abolished by ouabain, and therefore it is possible that a portion of the intestinal active transport of amino acids may not rely totally upon the Mg^{2+} dependent $Na^+ - K^+$ activated ATPase [16, 17, 18]. There is apparently no evidence as yet to suggest that this ouabain resistant transport system for amino acids also operates in the kidney.

None of these hypotheses, however, explains why the intestinal absorption of a broad cross section of amino acids is affected by lead poisoning, sodium maleate and malnutrition, whereas intestinal absorption of other amino acids is not.

It would appear therefore, that more work, using a wider selection of amino acids, needs to be carried out to further clarify amino acid transport patterns in a variety of situations such as lead poisoning, protein deprivation and also in magnesium depletion.

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C. H. R. Thirumalai, Department of Physiology, University of the West Indies, Mona, Kingston 7, Jamaica, West Indies