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Original article

Intestinal transfer of manganese: resemblance to and competition with calcium

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Summary — The effect of calcium, phosphate and the sugars lactose and sorbitol on the intestinal absorption of manganese were studied in adult male rats. Gastric gavage showed that lactose (100 mM or 200 mM) increased the hepatic retention of ^{54}Mn, while phosphate decreased it. *In situ* ileal loop studies indicated that Mn absorption was normally complete in 30 min. Sorbitol had no effect on uptake during this period, but extended Mn absorption from 30 min to 120 min. Low concentrations of Mn (10 μM) did not alter the enhancing effect of lactose on calcium transport (10 mM), but the enhancing effect of lactose on Mn transport was blocked by this high calcium concentration. Intestinal alkaline phosphatase activity was rapidly stimulated by Mn. These similarities plus the competition between cations, especially calcium, suggest that a common mechanism exists in their intestinal transport.

Mn / Ca / intestinal transfer / sugar / phosphate

Résumé — Absorption intestinale du manganèse : analogies et compétitions avec le calcium. *On étudie les effets de composés glucidiques, lactose ou sorbitol, du phosphate et du calcium sur l'absorption intestinale du manganèse :*
a) introduit par sonde gastrique, le lactose 100 ou 200 mmol.l^{-1} présent dans la solution 50 μmol.l^{-1} de manganèse, accroît la rétention hépatique du cation; celle-ci diminue en présence de phosphate;
b) l'absorption du Mn injecté en anse iléale in situ *:*
– est terminée en 30 min. À ce moment là, en présence de sorbitol, l'absorption se poursuit;
– à faible concentration (de l'ordre de 10 μmol.l^{-1}), Mn ne modifie pas l'effet d'augmentation du transport de Ca (10 mmol.l^{-1}) par le lactose (100 mmol.l^{-1}). Mais l'effet accélérateur du glucide sur le transport de Mn est fortement atténué par de fortes concentrations en Ca;
– plus rapidement que d'autres cations, Mn augmente l'activité de la phosphatase alcaline intestinale.
Ces analogies, et ces compétitions entre cations, impliquent particulièrement le calcium, suggèrent que quelque mécanisme commun est impliqué dans leur transport intestinal.

transfert intestinal / Mn / Ca / glucides / phosphates

INTRODUCTION

The intestinal transport of manganese seems very similar to that of other divalent cations, so much so that they could all be transported by a common mechanism. Two very different lines of research suggest a shared fate for cations during their intestinal absorption. The intestinal transport of a cation, such as lead, is decreased by the addition of other cations, such as calcium, zinc or iron (Manaffey, 1981). Although it has been less well studied, the behaviour of manganese in this respect is comparable. For instance, iron absorption is increased in isolated gut segments of iron-deficient rats, as is the absorption of cobalt, manganese and zinc (Forth, 1970). As has been shown for lead, calcium supplementation reduces manganese uptake, enhances its fecal excretion, and decreases its concentration in the blood and liver (Johnson and Kies, 1989). Mn absorption is inhibited by a high load of dietary calcium in several animal species (Hawkins *et al,* 1955; Van Barneveld and Van den Hamer, 1985; Smith and Kabaija, 1986) as in man (Greger *et al,* 1977; Freeland-Graves and Lin, 1991), but this effect has occasionally not been observed (Spencer *et al,* 1979). Manganese uptake by brush border intestinal vesicles is also markedly inhibited by iron, cobalt and zinc (Kabata *et al,* 1989). These observations seem to indicate that all these cations compete for a common site of transfer.

The second line of evidence is the effect of dietary sugars. The absorption of these cations, principally divalent, is greatly enhanced in the presence of sugars (Fournier and Dupuis, 1981; Bushnelli and De Luca, 1983); lactose and sorbitol have been the most extensively studied. They were found to enhance intestinal manganese transport by Fournier and Fournier (1972), while King *et al* (1980) reported that they depressed ^{54}Mn retention by rat tissues.

Lastly, a number of correlations have been observed between calcium transport, mainly intestinal, and alkaline phosphatase activity. This enzyme is particularly abundant and active where material transport is most intense, such as in the intestinal microvilli. The enzyme activity also seems to be dependent on cations, such as calcium (Hanna *et al,* 1979; Tardivel *et al,* 1987). The effect of manganese on intestinal phosphatase activity is not known. However, this cation activates alkaline phosphatases from bovine kidney (Cathala *et al,* 1975) and from various subcellular fractions of insect tissues (Hodgson and Kumar, 1964).

This study was performed to determine the following:

– whether there is a latency time before sugars stimulate intestinal Mn transport, as there is for calcium transport (Lengemann *et al,* 1959; Dupuis *et al,* 1980);

– the effects of sugar and phosphate on this transport, as these compounds act in opposite ways on intestinal calcium transport (Dupuis *et al,* 1977; Mc Dermott and Kies, 1987);

– whether there is competition between manganese and calcium with respect to the effect of sugars on their transport. Published results are contradictory. Lactose or milk markedly (Mc Dermott and Kies, 1987), or slightly (Freeland-Graves and Lin, 1991) decrease the inhibitory effect of high Ca load on Mn absorption;

– the effects of manganese on the catalytic activity of intestinal alkaline phosphatase.

MATERIALS AND METHODS

All experiments were carried out on adult male Wistar rats. They were fed *ad libitum* with a balanced diet containing 0.6% calcium, 0.375% phosphorus and 0.005% manganese (Randoin and Causeret, 1947). Daily ingestion of 20 g diet supplied an adult rat with ≈ 1 mg Mn.

Intestinal manganese transfer

Ileal loop experiments

Rats were fasted overnight, anesthetized with ether, and an ileal loop (12 cm from the ileocaecal junction) was isolated *in situ* by tying off the intestine. The loop was filled with 2 ml of the test solution containing ^{54}Mn; the composition of the latter is indicated for each experiment. The rats were killed at different times following injection. Each loop including the wall and the remaining test solution was removed and ^{54}Mn was measured in a gamma counter (17% yield). The transfer coefficient was calculated from the formula:

$$\frac{(\text{injected } ^{54}Mn - \text{intestinal } ^{54}Mn)}{\text{injected } ^{54}Mn} \times 100$$

Livers were removed and their ^{54}Mn determined. The livers were not washed, as ^{54}Mn was not measurable in the blood under these experimental conditions.

Stomach tube experiments

The rats were fasted for 18 h and then given 2 ml test solution by gavage. The rats were killed at different times following gavage. The livers were removed and ^{54}MN determined.

When the test solution contained Mn^{2+} and Ca^{2+}, calcium transfer was determined from plasma ^{45}Ca measured by liquid scintillation counting. In these experiments, only ^{45}Ca was measured in the plasma, as ^{54}Mn was not detected under these conditions. ^{54}Mn transfer was estimated by liver uptake.

Preparation of intestinal alkaline phosphatase

The intestine was isolated from the Treitz angle to the ileocaecal junction. The mucosa was scraped off, pooled and homogenized. Alkaline phosphatase was purified by a modification of the method of Saini and Done (1972); the main steps were butanol treatment, ammonium sulfate fractionation and DEAE–cellulose chromatography. The activity of the final preparation was 245 IU/mg protein.

Alkaline phosphatase activity

Alkaline phosphatase activity was determined at 37 °C in 3 ml 30 mM Na_2CO_3 – $NaHCO_3$ buffer, pH 9.6. The concentrations of paranitrophenylphosphate (PNPP), the different cations and the incubation time are given for each experiment.

Statistical analysis

The results are given as means ± SEM. The groups were compared by using Student's *t*-test.

RESULTS

Effect of sorbitol on ileal Mn transfer in the presence and absence of NaCl

Table I shows that Mn ileal transfer was not influenced by 0.9% NaCl. One hundred mM sorbitol significantly increased Mn transfer (60%) with or without NaCl. Thus the test solutions probably became rapidly isotonic (intestinal secretions) since lactose has been shown to stimulate Ca absorption whether or not the solution contains 0.9% NaCl (Wasserman and Lengeman, 1960).

Effect of sorbitol on the duration of Mn transfer (fig 1)

There was no significant difference between Mn transfer in the control and sorbitol groups at 30 min after instillation. Mn transfer did not change appreciably in control rats between 30–120 min. Mn content of the ileal loop (fig 1A) and Mn retention in the liver were found to be the same (fig 1B). However, ileal Mn transfer was signifi-

Table I. Effect of sorbitol on ileal manganese transfer, in the presence or absence of NaCl in the instilled solution.

	Control	*Sorbitol (100 mM)*	*Sorbitol effect in % of control*
H_2O	8.4 ± 0.7 [a]	13.2 ± 1.2	+ 69 ($P < 0.01$)
NaCl 0.9%	9.6 ± 1.1 [b]	14.8 ± 1.1 [b]	+ 54 ($P < 0.05$) [b]

Injection into the ileal loop of 1 ml of 50 μM $MnSO_4$ (+1 μCi ^{54}Mn) solution containing sorbitol 100 mM, H_2O or NaCl 0.9%. Rats were killed 2 h after injection. [a] Mean ± SEM for 6 12-month-old rats; [b] not significant with respect to the animals not receiving NaCl.

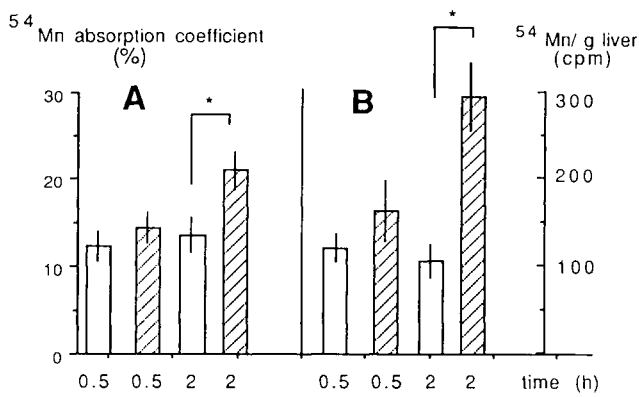

Fig 1. Time course effect of sorbitol on Mn ileal absorption (A) and Mn liver retention (B). One ml of a 50 μM $MnSO_4$ (+ 1 μCi ^{54}Mn), 0.9% NaCl solution containing 100 mM sorbitol (hatched column) or not (open column) was injected into lieal loops of 5 12-months-old rats. Results are mean ± SEM; * denotes a statistically significant difference ($P < 0.01$) between columns considered.

cantly enhanced (50%) between 30 and 120 min in the presence of 100 mM sorbitol (fig 1A). Similarly, sorbitol induced a significant increase in liver Mn between 30 and 120 min; this value was 3-fold that of controls at 120 min (fig 1B).

Effect of lactose or phosphate on intestinal Mn transfer (fig 2)

Mn transfer was estimated by determining ^{54}Mn in the liver 2 h after gavage with 50 μM $MnSO_4$ solution containing 1 μCi ^{54}Mn. Liver ^{54}Mn increased with lactose concentration of the gavage solution. Thus, 50 mM lactose increased Mn transfer by 19% (not significantly different from controls). 100 mM lactose increased transfer by 117% ($P < 0.01$) and 200 mM lactose by 251% ($P < 0.001$).

Phosphate (50 mM) had the opposite effect, significantly decreasing by the amount of Mn in the liver by 47% ($P < 0.02$).

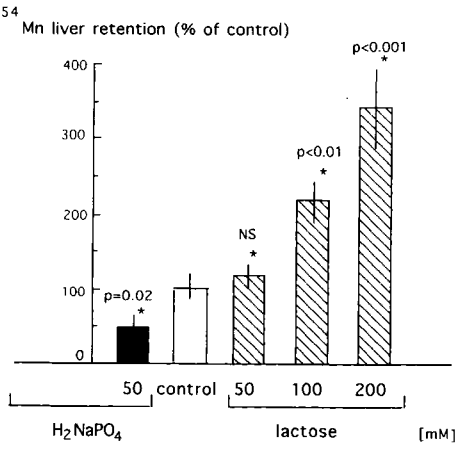

Fig 2. Effect of phosphate and lactose on ^{54}Mn liver retention (mean ± SEM). Two ml of a 50 μM $MnSO_4$ (+μCi ^{54}Mn) 0.9% NaCl solution were administered *via* stomach tube in the presence of either phosphate or lactose at indicated concentrations into 6 4-month-old rats for each group. The rats were killed 2 h after ingestion. ^{54}Mn liver retention was expressed relative to group control considered as 100; * denotes a statistically significant difference between the control group and the other groups.

Table II. Interaction between Mn and Ca intestinal transfer.

	^{45}Ca plasma (% ingested /100 ml)	*^{54}Mn in liver (% ingested)*
Exp 1		
Control	10.0 ± 1.1[a]	16.0 ± 1.9
100 mM lactose	16.1 ± 1.4[b]	8.7 ± 0.8[b]
Exp 2		
Control	7.2 ± 0.9	14.2 ± 1.3
200 mM lactose	17.6 ± 2.1[b]	19.3 ± 1.4[ns]

Ingestion *via* stomach tube of 2 ml of 20 μM $MnSO_4$ (+2 μCi ^{54}Mn), 10 mM $CaCl_2$ (+2 μCi ^{45}Ca), 0.9% NaCl solution with or without lactose. The rats were killed 2 h after ingestion. [a] Mean ± SEM for 5 4-month-old rats; [b] denotes a statistically significant difference between the control group and the study group; [ns] not significant with respect to the control group.

Interactions between the intestinal transfer of Mn and Ca

Calcium transfer was estimated from the plasma ^{45}Ca concentration, and Mn transfer from the Mn concentrations in the liver (table II). Mn (20 μM) did not modify the effect of lactose on calcium transfer when the calcium concentration in the ingested solution was high (10 mM). The plasma ^{45}Ca concentrations were similar to those observed in the absence of Mn, since 100 mM lactose enhanced ^{45}Ca uptake by 61% and 200 mM lactose enhanced it by 144%. A high Ca concentration (10 mM) in the ingested 100 mM lactose solution significantly decreased Mn transfer (–45.6%), whereas this same concentration of lactose enhanced Mn transfer (117%) in the absence of Ca (fig 2). Two hundred mM lactose had little effect on Mn transfer (35% increase) in the presence of Ca, but enhanced it by 251% in the absence of Ca (fig 2).

Effect of Mn and other bivalent cations on intestinal alkaline phosphatase activity (fig 3)

The initial rate of *p*-nitrophenylphosphate (PNPP) hydrolysis at low substrate concentrations was linear from 0.05–0.5 mM PNPP (incubation time = 10 min) in the absence of cations. The rate was doubled at each PNPP concentration in the presence of 1 mM Mg^{2+}. 1 mM Mn^{2+} was even more effective, quadrupling the hydrolysis rate at 0.25 mM PNPP. The effects of Mn and Mg were the same at 0.5 mM PNPP.

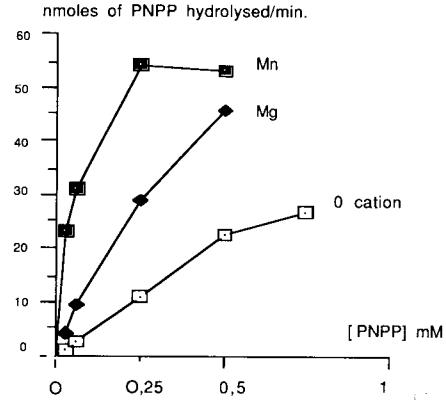

Fig 3. Intestinal alkaline phosphatase activity with different concentrations of PNPP. Effect of either 1 mM Mg or 1 mM Mn in the incubation medium. Incubation time: 10 min.

DISCUSSION

Several factors are known to affect manganese absorption, including species, age, sex and nutritional status (Lonnerdal *et al,* 1987; Lee and Johnson, 1988). The present study examined the effects of sugars on manganese transport, which is similar to calcium transport. It is thus possible that a common mechanism is implicated in all divalent cation transport.

The intestinal transfers of Ca^{2+} and Mn^{2+} are competitive. When the manganese concentration was 500-fold lower than that of calcium, calcium strongly blocked the enhancing effects of lactose on manganese transfer, although the enhancing effect of this sugar on calcium transfer was not influenced by manganese. On the other hand, when the concentrations of manganese and calcium were equimolecular, the enhancing effect of lactose on intestinal calcium transfer was suppressed (Fournier *et al,* 1972). Thus the relative concentrations of the cations determined their competitive action (Johnson and Kies, 1989; Freeland-Graves and Lin, 1991).

The mutual antagonism between calcium and manganese transfer has been interpreted in terms of their impact on calcium channels (Chiesi and Inesi, 1981), or interaction on a common transport site (Rosenberger and Triggle, 1978). It has also been shown that this cation inhibits iron uptake and release by rat liver slices (Saltman *et al,* 1956). It is possible that these phenomena only reflect the general property of proteins to take up and bind cations according to their respective concentrations and affinities for the proteins.

A second test measured the effect of phosphate and sugars on intestinal transfer and hepatic retention of manganese. The effect of sugars showed 2 similarities between manganese and calcium. Firstly, the effect of the sugar on the ileal transfer of manganese had a delayed onset, as previously observed for calcium. Secondly, as for calcium transfer, manganese transfer was enhanced by sugar and decreased by phosphate. The effects of sugars and phosphate on Mn transfer could indicate that they modify an enzymatic function. However, the decreasing action of phosphate on manganese transfer could result from precipitation of this cation. But the action of the sugar was correlated with an increase in intestinal calcium transfer and an increase in the transphosphorylase activity of intestinal alkaline phosphatase with increasing sugar concentration (Dupuis *et al,* 1991). Phosphate binding to the sugar could reduce the insolubility of some cations in the gut, thus increasing their absorption time.

Mg and Zn are the best activators of alkaline phosphatase, but Mn was a 2-fold better activator of intestinal alkaline phosphatase than Mg in our experimental conditions. This enzyme may be important for

intestinal cation transport. Its mineral-dependence, although non-selective, is also a feature of ATPases which are known to be important for the transport of their activating cations. Mn like Mg modulates Ca transport and ATPase activity in sarcoplasmic reticulum vesicles (Chiesi and Inesi, 1981). Moreover, phosphorylatable microvillar proteins display identical electrophoretic properties to the forms of alkaline phosphatase throughout the small intestine (Crouzoulon *et al,* 1983; De Jonge *et al,* 1981; Tardivel *et al,* 1987). Such protein phosphorylation is essential for transmembrane transport and its regulation.

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