The intestinal absorption of magnesium and lithium in the guinea pig

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Zusammenfassung

Die intestinale Absorption von Magnesium und Lithium wurde an dem nach *Lauterbach* isolierten Mucosa-Präparat untersucht. Die Kinetik der Mg-Absorption läßt auf ein aktives Transportsystem schließen, das bei luminalen Mg-Konzentrationen über 2 mmol gesättigt ist. Inhibitoren des Stoffwechsels reduzierten den Mg-Transport, ohne ihn aber völlig aufzuheben. Für Lithium ergab sich ein passiver Absorptionsprozeß; die Meßparameter sind charakteristisch für Diffusionsvorgänge.
Zusatz von Bumetanid auf der luminalen, nicht aber auf der Serosa-Seite, reduzierte den Mg-Transport signifikant.

Summary

The intestinal absorption of magnesium and lithium was investigated using the isolated mucosa *Lauterbach* preparation. Kinetics of magnesium absorption were indicative of an active system with saturation being observed at luminal magnesium concentrations above 2 mM. Metabolic inhibitors reduced but did not abolish magnesium transport. Lithium absorption was shown to be a passive process with parameters characteristic of diffusion.
Luminal but not serosal additon of bumetanide significantly reduced magnesium transport.

Résumé

On a étudié l'absorption intestinale du magnésium et du lithium au moyen du modèle de préparation de muqueuse isolée de *Lauterbach*. La cinétique de l'absorption magnésique a montré la présence d'un système actif, se saturant lorsque les concentrations luminales de magnésium étaient supérieures à 2 mM. Les inhibiteurs métaboliques ont réduit mais n'ont pas supprimé le transport de magnésium. L'absorption du lithium est apparue être un processus passif présentant les paramètres caractéristiques de la diffusion.
L'addition luminale, mais non séreuse, de bumetanide a réduit le transport de magnésium de façon significative.

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Introduction

The intestinal absorption of magnesium has been the subject of much controversy. Several mechanisms to describe magnesium absorption have been proposed. These include passive diffusion [1, 2], carrier mediated transport [14, 15] and a combination of carrier transport and diffusion [12, 13]. These previous studies have utilised several different experimental techniques with most workers using rat as the species of study.
The intestinal absorption of lithium has been previously studied in this laboratory using everted sacs of rat intestine [5]. Lithium absorption was found to be a passive process unaffected by metabolic inhibitors [6].
Experiments were performed using the isolated mucosal preparation of guinea pig jejunum [10]. This technique separates the absorptive epithelium from the muscle and connective tissue layers present in most in vitro preparations. These muscle and connective tissue layers are responsible for reduced transport and are the rate limiting layers in the absorption process [3]. Therefore, this technique is particularly useful in determining the kinetic profiles of transported compounds.
In the experiments described in this paper the kinetic analysis of magnesium and lithium absorption were undertaken and the effect of several drugs on magnesium absorption were studied.

Materials and Methods

Guinea pigs (strain Dunkin Hartley) between 450 – 600 g in weight of either sex were maintained on Oxoid 18 diet and tap water. Food was removed 18 hours prior to experiment but free access to water was permitted at all times.
The animal was maintained in a state of neuroleptanaesthesia throughout the experiment [7]. Premedication with atropine sulphate (0,06 mg s.c.) and sodium pentabarbital (30 mg/kg i.p.) leads to recumbency after approximately 15 minutes. The neuroleptanalgesic combination (droperidol 5 mg/kg and phenoperidine 1 mg/kg) is given i.p. The animal becomes completely analgesic and the operation may begin after a further 15 minutes. The anaesthetised animal was placed on a preheated pad and maintained at 37 °C. Heart rate and rectal temperature were recorded on a four channel flat bed recorder and were shown to remain within the normal range throughout the experimental period.

The animal was opened by midline incision and the small intestine exposed. The jejunum was located and any intestinal contents were gently flushed out using 20 cm^3 of incubation media with the following composition (mM): 136,NaCl; 7,7,KCl; 1,1,$MgSO_4$; 1,1,$CaCl_2$; 1,0 Na phosphate buffer pH 7,4; 1,0 Tris pH 7,4; 14,0, glucose; 14,0, mannitol.

Sequential pieces of intestine (1 – 2 cm in length) were removed with the blood supply being maintained intact until immediately prior to excision.

Bleeding was prevented by careful use of clamps.

The tissue was opened lengthwise and the muscle and connective tissue layers were separated from the mucosal layer using a razor blade and a ground glass plate. The epithelium was then supported on a nylon mesh and clamped between two flux chambers where it formed a separating membrane occluding a porthole of known area. Incubation media of required composition was introduced onto either side of the membrane. The total tissue preparation time did not exceed one minute. The chamber was placed into a water bath at 37 °C and the bathing solutions were simultaneously mixed and oxygenated using 100 % O_2. At the end of the incubation period (45 minutes) the solutions were removed and assayed. A specially designed tissue punch allows removal of the area of the epithelium in contact with the bathing solutions.

In incubation solutions magnesium chloride or lithium chloride was substituted for sodium chloride up to a maximum of 50 mM.

Structural viability, or the presence of artificial holes in the membrane as a result of the stripping procedure was checked by using the extracellular marker ^3H polyethylene glycol 900 (PEG 900) placed on one side of the membrane [8]. Any chamber in which PEG-900 permeability was greater than 0,6 % was rejected as being leaky. Glucose transport and lactate dehydrogenase release were also studied as measures of tissue viability.

The magnesium and lithium content of the buffers was determined by AAS. Tissue concentrations were obtained by dissolving known masses of tissue in a quaternary ammonium tissue solubilizer (NCS, Amersham). This was then diluted to volume and again assayed by AAS.

Materials

Droperidol and phenoperidine were generous gifts of Janssen Pharmaceuticals. Bumetanide was donated by Leo Laboratories. ^3H Polyethylene glycol 900 was supplied by NEN DuPont. All gases were supplied by British Oxygen PLC. All other materials were of Analar grade supplied by Sigma.

Statistics

Significance of differences between means was determined by Students-t-test. Correlations of points to a line were determined by the method of least squares. Significances of 5 % or less were taken as significant.

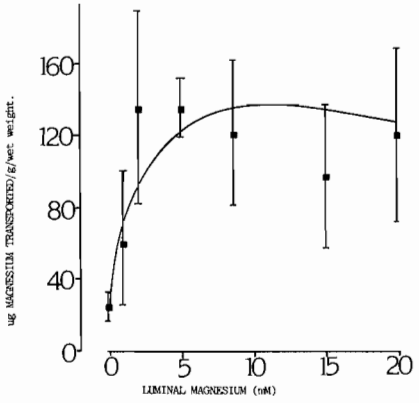

Fig. 1: Kinetic plot of magnesium absorption in guinea pig jejunum each value is mean of at least eight observations + standard deviations

Tab. 1: Viability tests of jejunal sheets. Glucose transport assayed by the peroxide method and LDH by automated assay

Glucose transport (S/M ratio)	1,4 + 0,3(10)	
Lactate dehydrogenase realease %	Serosal 0,7 + 0,1	Mucosal 0,9 + 0,1

Results

Viability

Isolated epithelia from guinea pig intestine were obtained for transport studies. Glucose transport with a serosal to mucosal ratio greater than unity was observed indicating a viable glucose transport system (Tab. 1). Lactate dehydrogenase (LDH) release from the isolated mucosa was determined.

Less than 1 % of the total tissue LDH was released in serosal or mucosal directions over the 45 minute incubation period (Tab. 1). Histological examination of the mucosa indicated no significant damage to the tissue after a 45 minute incubation (data not shown). These data indicate the mucosal sheets used experimentally were viable.

Transport Studies

The transport of magnesium and lithium in the luminal to serosal direction was studied.

Magnesium absorption was seen to saturate at luminal magnesium concentrations above 2 mM (fig. 1). In contrast, lithium absorption followed linear kinetics with no evidence of saturation being observed up to 20 mM (fig. 2).

Tissue magnesium concentrations did not significantly change with increasing luminal magne-

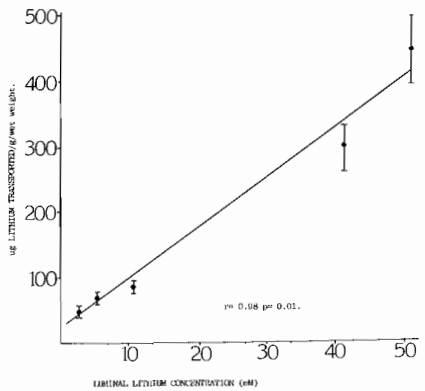

Fig. 2: Kinetic plot of lithium absorption in guinea pig jejunum. Each value is mean of at least five observations + standard deviations

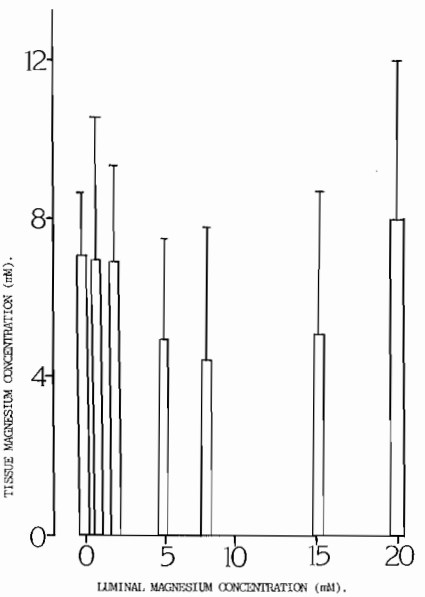

Fig. 3: Histogram showing jejunal tissue magnesium concentration with increasing luminal magnesium. Each value is mean of at least eight observations + standard deviations

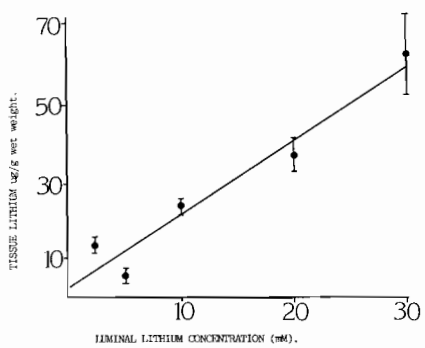

Fig. 4: Tissue lithium concentration with increasing luminal lithium. Each value is the mean of at least five observations + standard deviations

Tab. 2: The effect of cyanide, 2,4-Dinitrophenol, bumetanide and ouabain on jejunal magnesium concentrations and magnesium transport. Data are means + standard deviations of the number of observations in parentheses

Inhibitor	Tissue Mg [mM]	Mg Transport ug Mg/g/wet wt.
Control	6,11 + 3,17 (8)	169 + 25 (8)
Cyanide (2 mM)	3,41 + 1,48* (8)	112 + 26** (8)
2,4-Dinitrophenol (2 mM)	2,34 + 0,74* (8)	126 + 29** (8)
Bumetanide (1 mM)	5,41 + 1,26 (4)	113 + 48* (8)
Bumetanide (ImM serosal)	5,73 + 1,78 (4)	175 + 93 (4)
Ouabain (1 mM serosal)	6,15 + 0,73 (5)	136 + 32* (8)

* = $p > 0,05$
** = $p > 0,01$

sium concentration (fig. 3), whereas tissue lithium concentrations showed a significant correlation with luminal lithium (fig. 4).
Magnesium efflux from the intestinal tissue in the normal state was significantly greater in the luminal direction than in the serosal direction (fig. 5).
No significant correlation was obtained between magnesium transport and intestinal permeability as measured by PEG 900 (fig. 6). However, a significant correlation between lithium transport and PEG 900 permeability was obtained (fig. 6).

Inhibitor Studies

The effect of several metabolic inhibitors and drugs on magnesium transport was studied. All compounds tested significantly reduced but did not abolish magnesium transport (Tab. 2).
Luminal but not serosal bumetanide caused a significant reduction in magnesium transport (Tab. 2) and serosal but not luminal addition of ouabain significantly reduced magnesium transport (Tab. 2).
Ouabain and bumetanide did not significantly affect tissue magnesium concentrations (Tab. 2).
The other metabolic inhibitors used in this study significantly reduced tissue magnesium concentrations (Tab. 2).

Discussion

The data obtained indicate that functional intact mucosal sheets

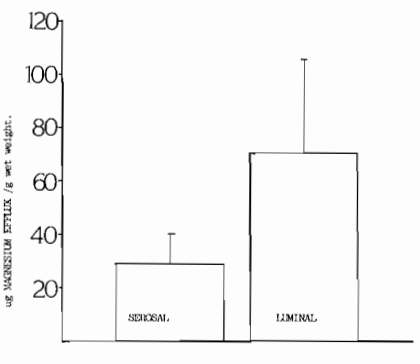

Fig. 5: Efflux of magnesium from jejunal tissue in luminal and serosal directions at 45 minutes. Each value is the mean of 18 observations + standard deviations

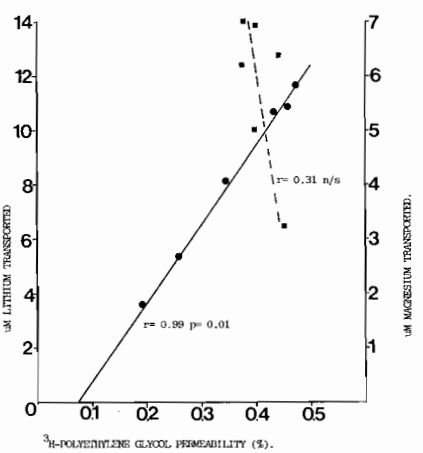

Fig. 6: The correlations between magnesium ■ and lithium ● transport and ^{3}h-polyethylene glycol permeability

were used in these studies. Magnesium transport from intestinal lumen to blood was seen to be a complex system with kinetics indicating a saturation of absorption at luminal magnesium concentrations above 2 mM. This observation is in agreement with the data of several previous workers [12, 13] who indicated transport systems being composed of an active and passive component.

In experiments using cyanide and 2,4-dinitrophenol as metabolic inhibitors magnesium transport was reduced. This data suggests metabolic energy is required for magnesium transport.

Metabolic inhibitors also caused significant reductions in tissue magnesium concentrations. From these experiments we can say that in the guinea pig magnesium transport is partially sensitive to metabolic inhibitors and therefore has an active transport system.

Despite the use of metabolic inhibitors magnesium absorption is not halted. However, this residual transport may be due to metabolic inhibitors causing intracellular magnesium efflux.

The effect of bumetanide on magnesium absorption was investigated. Luminal addition of bumetanide reduced magnesium transport to a level comparable to that observed with metabolic inhibitors. However, it is important to note bumetanide did not cause a significant reduction in tissue magnesium concentrations. This suggests that bumetanide exerts a specific effect on magnesium transport and does not exert a gross antimetabolic effect. These observations are supported by the data obtained from the experiments where bumetanide was added to the serosal membrane of the epithelial sheet. Serosal bumetanide has no effect on magnesium transport or on tissue magnesium concentrations. This observation of an effect of bumetanide when added to the luminal bathing media suggests that the active magnesium transport system is present in the brush border membrane. Bumetanide sensitive magnesium transport has been reported previously in isolated cells [9].

The effect of the sodium pump inhibitor ouabain on magnesium transport was studied.

Blood side addition of ouabain significantly reduced magnesium transport but did not reduce tissue magnesium concentrations. The mechanism responsible for this effect remains unknown but it is possible the effect is due to abolition of the favourable electrochemical gradient (created by the sodium pump) for magnesium transport. This effect has also been observed in sheep rumen [11].

Lithium has been shown to have effects on magnesium dependent processes [4]. However, lithium absorption in contrast to magnesium showed no evidence of saturation in the present study. This indicates lithium absorption is a passive process which is in agreement with other data obtained in this laboratory using everted sacs of rat intestine [5, 6].

Conclusion

These studies indicate that the intestinal absorption mechanisms for lithium and magnesium are fundamentally different. Magnesium transport has an active component which is sensitive to metabolic inhibition and luminal addition of bumetanide. A second component of magnesium transport may be a diffusion process perhaps involving diffusion across the epithelial sheet via a paracellular route and along a favourable electrochemical gradient. Lithium absorption appeared to be by a simple diffusion mechanism.

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