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AN EFFECT OF PENETRATING IONS ON MAGNESIUM EFFLUX FROM RAT SMALL INTESTINE, *IN VITRO*

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SUMMARY

Incubation of isolated intestinal segments in varying concentrations of sodium, potassium, and lithium showed magnesium-ion displacement from the mucosal cells during and in the absence of glucose absorption. The extent of this displacement appears to be a function of monovalent cation entry into the epithelial surface as no loss of magnesium occurred when relatively non-permeable substitutes for electrolytes were used. It is suggested that a possible role of penetrating ions on the intestinal epithelium may be the unbinding of membrane magnesium for participation in regulating passive permeability. Magnesium unbinding may also be associated with activation of mucosal ATPase, possibly necessary for increased activity of the sodium pump.

INTRODUCTION

The requirement of the small intestine for sodium ions in the absorption and transport of glucose has been well established[1,2]. It has been suggested that this requirement involves sugar entry across the mucosal epithelium *via* a mobile carrier common to both sodium and glucose[3]. Other investigators have presented evidence which would tend to link the sodium dependency for the entry of glucose, or certain glucose analogues, to ATPase activation rather than a specific carrier component[4].

In our laboratory studies are being conducted concerning the effect of sugar entry into the mucosal epithelium on various mucosal electrolyte concentrations and their distribution along the small intestine. This report deals specifically with the apparent unbinding of magnesium ion from the mucosa of isolated everted sacs of rat small intestine during periods of monovalent cation penetration. Sugar-absorption data were not emphasized as the sugar used in the incubation media was of a concentration sufficiently high to obscure any direct sodium–glucose dependency at a carrier site. However, by using high glucose concentrations, maximal sodium turnover during absorption could be expected with a concurrent effect on other mucosal electrolytes.

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METHODS

The everted-sac method of CRANE AND WILSON was used for studies of glucose entry and net ionic flux. This procedure, with our modifications, has been previously described[5,6]. Male albino rats (Holtzman) of approx. 300 g were fasted 24 h with water *ad libitum* prior to each experiment. Ether was used for anesthesia and the entire small intestine removed, everted, and divided into 8 equal segments. This method of division allowed a comparison of alternate segments (*i.e.*, 1 *vs.* 2, 3 *vs.* 4, *etc.*) from the same animal. Sugar absorption under each experimental condition could also be compared on a segment-by-segment basis as a function of the mucosal media employed. To study the absorption of glucose by the intestinal mucosa, solutions containing sodium, potassium, or lithium ion, or relatively non-penetrating osmotic substitutes were used as follows: For Segments 1, 3, 5 and 7 the mucosal medium contained 300 mg% glucose (16.65 μmoles/ml) in iso-osmotic (300 mosmoles) mannitol, while Segments 2, 4, 6 and 8 were also incubated in 300 mg% glucose but with various concentrations of sodium ion balanced osmotically with appropriate amounts of mannitol. Potassium and lithium substitutions were always at concentrations assuring isotonicity. Maximal absorption for the sacs was established at all 8 levels by incubating equivalent segments in Krebs–Ringer bicarbonate solution containing the same glucose concentration. Serosal solutions for all experimental procedures consisted of iso-osmotic mannitol. All mixtures described were gassed with 5% CO_2 in O_2 for the duration of the incubation period. Initial and final volumes of the mucosal solutions were established gravimetrically, and final net flux of sodium and magnesium into or out of the tissue was established on samples of the solutions using a model 303 Perkin-Elmer atomic absorption spectrophotometer. Glucose absorption was determined by a glucose-oxidase method previously described[7]. For each solution, osmolarity was measured before and after incubation by a Precision Systems Osmette. Finally, recovery of the tissue from certain experimental conditions was determined by removing the segments from their respective incubation media and subjecting them to a second incubation in Krebs–Ringer bicarbonate solution containing 300 mg% glucose. If the segments along the intestinal length re-established a typical glucose-absorption gradient from the upper to lower intestine, recovery was assumed even though the magnitude of sugar absorption was depressed below control levels.

RESULTS

The effect of reduced sodium ion in the tissue bathing medium (*i.e.* mucosal solution) upon sugar uptake by the intestinal epithelium may be seen in Figs. 1 and 2. Incubation in osmotically valanced mannitol did not prevent glucose absorption, though the characteristic gradient along the intestinal length previously reported by us, and others, was no longer evident. It should be emphasized that this experiment consistently resulted in a net efflux of sodium ion from the incubating segments so that the final mucosal medium was not completely sodium-free. Analysis of final sodium content established mean values exceeding 0.5 mequiv/l for all 4 segmental levels. It is obvious that all sodium-free experiments were only such in terms of initial concentration.

No marked increase in sugar absorption was evident when the initial incuba-

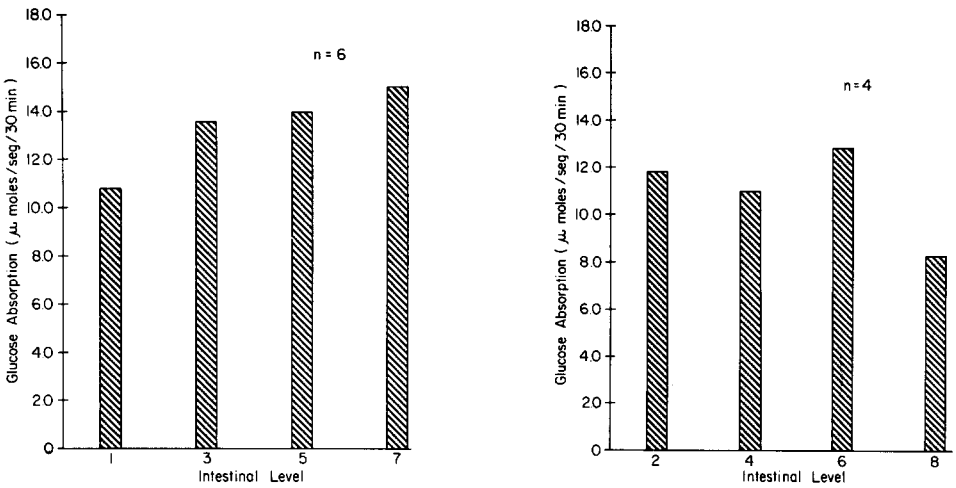

Fig. 1. Glucose absorption from mucosal solution at 4 alternate segments of rat small intestine. Incubation medium was iso-osmotic mannitol containing 300 mg% glucose (*i.e.* initially sodium-free). At 30-min incubation time Segment 1 contained 0.64 mequiv/l sodium; Segment 3, 0.52 mequiv/l; Segment 5, 0.55 mequiv/l and Segment 7, 0.50 mequiv/l.

Fig. 2. Glucose absorption from mucosal solution at 4 alternate segments of rat small intestine. Incubation medium was iso-osmotic mannitol in saline (Na^+ = 50 mequiv/l) containing 300 mg% glucose. At 30-min incubation time Segment 2 contained 49.4 mequiv/l sodium; Segment 4, 47.5 mequiv/l; Segment 6, 47.9 mequiv/l; and Segment 8, 46.3 mequiv/l.

tion medium contained increased sodium concentration (50 mequiv/l), as may be seen in Fig. 2. At the four intestinal levels there was a consistent net influx of sodium ion. This observation was least evident in Segments 2, 4, and 6 and maximal in Segment 8 which corresponds to the terminal ileum.

The consequence of sodium influx upon magnesium loss from the tissue during incubation may be seen in Fig. 3. The movement of magnesium ion from the tissue into the mucosal bathing medium seems to be directly related to sodium entry. Sodium uptake by an intestinal segment increased the appearance of magnesium in the mucosal solution 2- to 3-fold. In the sodium-free experiments consistent, but very small, amounts of magnesium were lost from the tissue. To determine if the efflux of magnesium was sodium-ion specific, substitution of sodium by either potassium or lithium was effected. It has been shown by others that neither potassium nor lithium can replace sodium for the mucosal accumulation of certain glucose analogues against a concentration gradient in hamster intestinal mucosa. Potassium has been reported unable to support the entry of these analogues across the mucosal brush border, while lithium apparently will allow a greater equilibration of these sugars between the intra- and extra-cellular compartments[2]. If the uncoupling of magnesium observed in the sodium experiments was directly related to sugar transport, one would expect differences to be observed in this regard with the lithium, potassium substitutions. The results of these experiments may be seen in Table I. No statistical differences could be demonstrated between magnesium loss from the mucosa and the specific electrolyte used either in the presence or absence of glucose from the bathing medium.

Osmolarity was determined for both initial and final mucosal solutions under

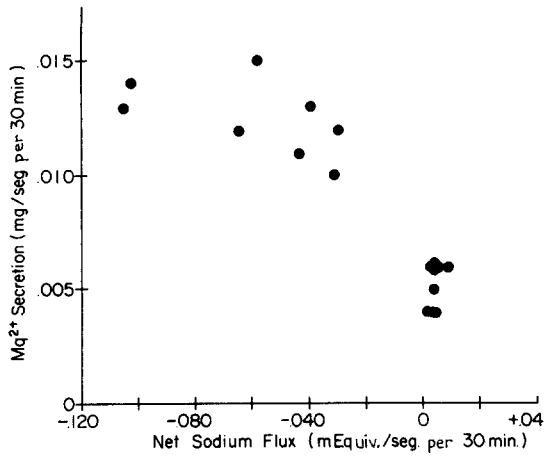

Fig. 3. Effect of sodium penetration on magnesium efflux from intestinal mucosa. Each point represents total efflux of magnesium ion from 4 segments (2, 4, 6 and 8) of rat small intestine as a function of sodium ion. Negative (−) flux = sodium loss from mucosal solution, and positive (+) flux = sodium appearance in mucosal solution. All incubation periods were of 30-min duration at 37°. Positive (+) flux occurred in initially sodium-free solutions, while negative (−) flux occurred as a function of initial solutions containing sodium.

all experimental procedures, and in no case was the final mean osmolarity different from the initial by more than 5 mosmoles.

This observation must be considered in terms of both electrolyte movement (into and out of the tissue) and fluid absorption. Table II shows the relative fluid movement as it is related to the presence and absence of the various electrolytes in

TABLE I

COMPOSITION OF MUCOSAL MEDIUM RELATED TO Mg^{2+} FLUX DURING INCUBATION OF EVERTED INTESTINAL SACS

*Medium***	*Mean Mg^{2+} secretion (mequiv × 10^3 per 30 min)*			
	NaCl	*KCl*	*LiCl*	*Mannitol*
Segment 1*	1.0	1.4	1.0	0.6
Segment 2	1.1	1.4	1.0	
Segment 3*	1.0	1.2	1.1	0.4
Segment 4	1.0	1.2	1.1	
Segment 5*	1.0	1.1	1.1	0.4
Segment 6	1.0	1.1	1.1	
Segment 7*	0.9	0.9	1.0	0.4
Segment 8	1.1	1.0	0.9	
Mean	1.0	1.2	1.0	0.4
Range	0.8–1.5	0.8–1.6	0.9–1.4	0.2–0.8
n	58	37	39	24

* Mucosal medium for Segments 1, 3, 5 and 7 contained 300 mg/100 ml glucose.
** All mucosal solutions shown were isotonic (300 mosmoles/l).

TABLE II

FLUID UPTAKE BY EVERTED INTESTINAL SEGMENTS DURING INCUBATION AS RELATED TO COMPOSITION OF MUCOSAL MEDIUM

*Medium***	*Fluid uptake (ml/30 min)*			
	NaCl	*KCl*	*LiCl*	*Mannitol*
Segment 1*	0.17	0.10	0.11	0.34
Segment 2	0.11	0.11	0.08	
Segment 3*	0.15	0.09	0.09	0.44
Segment 4	0.10	0.09	0.10	
Segment 5*	0.10	0.09	0.07	0.47
Segment 6	0.10	0.12	0.16	
Segment 7*	0.17	0.09	0.08	0.50
Segment 8	0.08	0.10	0.09	
Mean	0.12	0.10	0.10	0.44
Range	(0.00–0.28)	(0.00–0.20)	(0.00–0.28)	(0.17–0.74)
n	60	37	38	32

* Mucosal medium for Segments 1, 3, 5 and 7 contained 300 mg/100 ml glucose.
** All mucosal solutions shown were isotonic (300 mosmoles/l).

the initial mucosal solution. In all cases the penetration (net influx) of a monovalent ion was accompanied by small amounts of fluid absorption. When electrolyte-free mucosal solutions were used, the absorption of water was increased by large values.

DISCUSSION

Previous attempts to determine the requirement of sodium ion for sugar transport have shown conflicting results. For example, it has been reported that rat intestine perfused *in situ* with a 3-*O*-methylglucose–mannitol solution gave no evidence of sodium leakage into the lumen[4]. This observation has presented a question in regard to the sodium–glucose carrier concept, and has strengthened the possibility of a sodiun intracellular action in the glucose-transport phenomenon. However, the substrate concentrations were quite high and may have obscured the role of sodium shown by others using different glucose analogues. The results presented in this paper, obtained from isolated intestinal segments, indicate that during mannitol–glucose absorption experiments small, but perhaps significant, amounts of sodium ion became available to the mucosal surface during a 30-min incubation period. In addition, increasing the initial sodium concentration from approx. 0.0 to 50.0 mequiv/l does not markedly increase the uptake of glucose by any level of the rat small intestine at the substrate concentration employed. Consistently, the net entry of sodium into the mucosa increased when the external bathing medium contained increasing amounts of sodium ion. This uptake of sodium (measured as disappearance from the mucosal solutions) was always accompanied by loss of mucosal magnesium to the incubating medium. Several explanations for this occurrence are possible, among which one must consider the displacement of intracellular bound magnesium by penetrating sodium ion. If, as has been reported, mucosal membrane ATPase is dependent upon magnesium for activation, displacement may be involved in this process. It seems unlikely that the

penetration of sodium and the loss of magnesium can be accounted for by non-selective membrane permeability changes as several measurements of potassium efflux showed no obvious increase in its loss from the tissue.

Our data indicates the presence of glucose is not necessary for magnesium loss from the mucosa during incubation. However, it is obvious that this does occur as a function of electrolyte concentration in the bathing medium. As no real differences in this regard were observed with the three monovalent cations studied it is probable that magnesium uncoupling during incubation is a phenomenon not directly related to sugar absorption or transport. This conclusion does not rule out the possibility of a more indirect relationship for magnesium mobilization and passive transport of sugar through the membrane phospholipid component. Similar observations utilizing artificial phospholipid membranes have recently been reported[8].

Large increases in water absorption were observed in experiments utilizing mannitol and glucose. As no significant alterations in the osmolarity occurred between the initial and final mucosal solutions it must be concluded that mannitol was entering the mucosal tissue iso-osmotically in conjunction with glucose. The addition of monovalent ion reduced the fluid movement to values consistent with or below those obtained when balanced Krebs-Ringer solution was used. It is recognized that this observation is in direct contrast to that found by others using a different mammalian species[9], but has been verified for the rat intestine numerous times in our laboratory.

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