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THE EFFECT OF LITHIUM ON INTESTINAL SUGAR TRANSPORT*

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SUMMARY

1. The sugar uptake of hamster small intestine *in vitro* was measured with various replacements for Na^+ in the medium. None of them supported transport against a concentration difference, but sugar equilibration was significantly more rapid in the presence of Li^+ than of mannitol, choline or K^+.

2. This stimulatory effect of Li^+ was inhibited by 0.5 mM phlorizin and was observed with 3-*O*-methyl-D-glucose, D-galactose and α-methyl-D-glucoside, which are all actively transported in the presence of Na^+, but not with L-fucose and L-arabinose. Mutual inhibition between 3-*O*-methyl-D-glucose and D-galactose was observed in Li^+ medium but not in mannitol medium.

3. Li^+ had no effect on the entry of α-aminoisobutyric acid.

4. It is suggested that Li^+ stimulates sugar entry by activating the sugar "carrier" and not by interacting with the Na^+ pump. These findings are consistent with the hypothesis of CRANE *et al.*[3,4] that intestinal sugar active transport is mediated by an ion-activated carrier.

INTRODUCTION

Active transport of sugar in the small intestine has been shown to be dependent on the presence of Na^+ at the luminal surface of the epithelial cells[1] and on the operation of a Na^+ pump[2]. It was also found[2] that rapid, Na^+-dependent sugar entry continues when energy-dependent accumulation against a concentration difference is inhibited. These findings form the basis of a hypothesis by CRANE and co-workers[3,4], that the passage of sugar in either direction across the cell membrane is activated by Na^+, and that accumulation against a concentration difference is a consequence of the asymmetrical distribution of ions maintained by the Na^+ pump. On the other hand,

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CSÁKY[5] suggested that entry is not dependent on Na^+ but is followed by Na^+-dependent accumulation linked to the Na^+ pump in an as yet unspecified manner. Until now ionic activation of the entry step could only be demonstrated when sugar accumulation was prevented by inhibition of the energy supplied to the Na^+ pump. It would be desirable to be able to study this process without gross interference with metabolic processes.

When Na^+ in the intestinal lumen was replaced by other substances, none was able to support active transport, but differences in rates of sugar entry[2,6,7] suggested that Li^+ may have a small stimulating effect. However, as shown by CLARKSON AND ROTHSTEIN[7], Li^+ seems not to be transported by the intestinal Na^+ pump in the rat. These observations suggest that Li^+ may affect sugar entry, but does not interact with the Na^+ pump in the intestine, and may serve, therefore, as a tool with which to study sugar entry separately from active accumulation. The present experiments were designed to examine this possibility.

METHODS

The experiments were done *in vitro* on transversely cut segments of hamster small intestine according to the procedure of CRANE AND MANDELSTAM[8]. The tissue was incubated with gentle shaking at 37° in an atmosphere of O_2–CO_2 (95:5, v/v). The basic incubation medium consisted of 120 mM NaCl buffered with 25 mM Tris chloride–bicarbonate (pH 7.4) and contained, in addition, the same concentrations of K^+, Ca^{2+}, Mg^{2+}, SO_4^{2-} and phosphate as the original KREBS–HENSELEIT[9] bicarbonate buffer with which it was isosmotic. Modified media were prepared by replacing the NaCl with isosmotic amounts of LiCl, KCl, choline chloride or mannitol, subsequently referred to as Li^+, K^+, choline$^+$ and mannitol media, respectively. The media also contained a mixture of a ^{14}C- or ^3H-labelled and unlabelled sugar to give the desired initial concentrations. Apparent extracellular space was determined with tracer amounts of mannitol[10], labelled with the isotope not added with the sugar. In all experiments the tissue was preincubated for 10 min in the basic medium and then rapidly transferred to fresh medium, containing the additions indicated for each experiment and incubated for 40 min. Samples of medium and tissue homogenates were deproteinized by the method of SOMOGYI[11], except in D-galactose experiments. In these, 5% trichloroacetic acid was used to avoid precipitation of phosphorylated metabolites of the sugar. After centrifugation, the radioactivity of supernatants was determined by double-label liquid-scintillation spectrometry[12] and the Li^+ content by flame photometry.

3-Methylglucose was a generous gift of Dr. W. L. Glen, Ayerst Research Laboratories. All other chemicals were obtained from commercial sources.

The results are expressed as "percent filling", *i.e.*, the concentration of sugar in intracellular water as a percentage of the final concentration in the incubation medium. The average tissue water content after incubation in each medium was determined separately and was corrected for the mannitol space measured in each tissue sample. Statistical evaluation was by Student's t test applied to paired observations within each experiment (pooled tissue of 2–3 animals).

RESULTS

The water content and the mannitol space of tissues incubated in the different media are summarized in Table I. The total water content was unchanged from the Na^+ control by the Li^+ medium, but was somewhat lowered by the mannitol and choline$^+$ media and increased by the K^+ medium. The mannitol spaces after incubation in Li^+, mannitol and choline$^+$ media were equal, and higher than those of tissues incubated in the Na^+ and K^+ media. These differences are qualitatively similar, but smaller than those found by others.

When fresh tissue is first put into a Li^+ medium, the relatively high Na^+ content of the extracellular water will influence sugar entry. For this reason the tissue was

TABLE I

WATER CONTENT AND MANNITOL SPACE OF HAMSTER INTESTINAL SEGMENTS AFTER INCUBATION IN VARIOUS MEDIA FOR 40 MIN AT 37°

Figures are means ± S.E. of the mean for 6 experiments.

Medium	*% of water*	*Mannitol space*
Krebs bicarbonate buffer	81.5 ± 0.8	20.6 ± 1.0
Li^+ medium	81.7 ± 0.7	33.4 ± 1.6
Mannitol medium	78.6 ± 0.7	33.7 ± 0.8
Choline$^+$ medium	78.5 ± 0.6	33.2 ± 1.8
K^+ medium	82.8 ± 0.8	23.2 ± 1.2

preincubated in a Li^+ medium (see METHODS section). Table II shows that during preincubation much Na^+ is lost from the tissue and replaced by Li^+. The Na^+ released during 10 min of preincubation produced a Na^+ concentration in the previously Na^+-free medium of about 10 mM; during the following 40 min the release of Na^+ and its replacement by Li^+ continued more slowly. The results in Table II are expressed on the basis of total tissue water because the distribution of Na^+ between the medium, and the extracellular and intracellular space is not known. No similar experiments were done with other media, but for uniformity the identical preincubation procedure was followed in all cases.

TABLE II

CONCENTRATION OF Na^+ AND Li^+ IN HAMSTER INTESTINAL SEGMENTS INCUBATED AT 37° IN Li^+ MEDIUM FOR 10 MIN OR PREINCUBATED FOR 10 MIN AND INCUBATED IN FRESH Li^+ MEDIUM FOR AN ADDITIONAL 40 MIN

Figures (mmoles per l of total tissue water) are means ± S.E. of the mean for 6 experiments.

Conditions	*Na^+*	*Li^+*
Fresh tissue	71.3 ± 2.4	0
10 min incubation	31.2 ± 0.9	58.0 ± 1.3
10 min preincubation and 40 min incubation	9.1 ± 0.9	70.4 ± 1.9

Fig. 1 compares the transport of 3-methylglucose in different incubation media and the effect of phlorizin thereon. 3-Methylglucose is a non-metabolized glucose analogue which is known to be actively transported in the intestine[13]. Phlorizin is believed to act as competitive inhibitor of sugar entry[14] and does not affect cell metabolism at the concentrations used here. A period of 40 min of incubation is longer than desirable for close approximation of initial transport velocities but was found necessary to give tissue concentrations high enough for accurate measurement. Thus, the data may serve as qualitative indications of relative rates of sugar entry although they tend to underestimate them. The results in Fig. 1 confirm earlier observations[2,6]

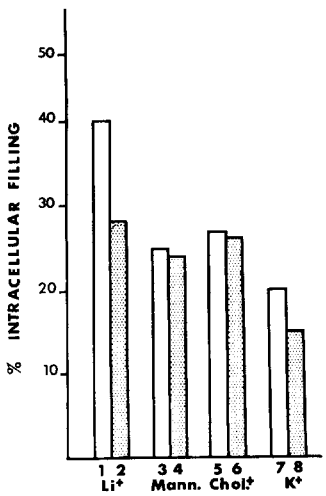

Fig. 1. Entry of 3-*O*-methyl-D-glucose in different media. Hamster intestinal segments were preincubated at 37° for 10 min and incubated for an additional 40 min in fresh medium, containing 0.5 mM sugar and tracer amounts of mannitol. The columns are the means of 6 experiments and represent intracellular filling as a percentage of sugar concentration in the incubation medium. Open columns in the absence and shaded columns in the presence of 0.5 mM phlorizin. The abbreviations under the columns refer to the type of medium used. Column 1 is significantly higher than all others ($P < 0.0025$); Column 7 is significantly lower than 3 and 5 ($P < 0.05$) but higher than 8 ($P < 0.005$).

that, in the absence of Na^+, actively transported sugars enter the tissue slowly. They show, however, that there are statistically significant differences in sugar entry depending on what replacement for Na^+ is present. Entry in Li^+ medium was significantly faster than in mannitol and choline$^+$ media and entry in K^+ medium was slower. These differences suggest that, in the absence of Na^+, these ions have effects of their own on sugar entry. The data on phlorizin inhibition serve to emphasize these differences: 3-methylglucose entry in Li^+ medium was significantly reduced by phlorizin, but entry in mannitol and choline$^+$ media was not significantly reduced. The effect of phlorizin in the K^+ medium was also significant.

If the stimulation of sugar entry by Li^+ is due to activation of the specific entry process (and not to a non-specific effect), it should be restricted to sugars entering by this process, *i.e.* which are actively transported[15] in the presence of Na^+. To test this prediction experiments were done to compare the entry in Li^+ and mannitol media,

TABLE III

ENTRY OF DIFFERENT SUGARS IN Li^+ AND MANNITOL MEDIA

Hamster intestinal segments were preincubated at 37° for 10 min and incubated for an additional 40 min in fresh medium containing 0.5 mM of sugar and mannitol tracer. Figures are means of 6 experiments and express intracellular filling as percentage of sugar concentration in the incubation medium. Li^+ and Mann. refer to Li^+ and mannitol media, respectively; Phl. refers to the addition of 0.5 mM phlorizin.

Sugar	*% of filling*				*Differences*	
	Li^+ + Phl. *a*	*Li^+* *b*	*Mann.* *c*	*Mann. + Phl.* *d*	*b − a*	*b − c*
3-*O*-Methyl-D-glucose	28	40	25	24	<0.005	<0.005
α-Methyl-D-glucoside	25	67	43	26	<0.005	<0.005
D-Galactose	20	51	33	20	<0.005	<0.005
L-Arabinose	33	36	35	32		
L-Fucose	33	32	36	35		

in the presence and absence of phlorizin, of five sugars, the first three of which are actively transported and the last two are not. It is readily apparent from Table III that, when compared to the mannitol control, Li^+ stimulated the entry of all three actively transported sugars but not of the two "non-transported" ones, and that in-

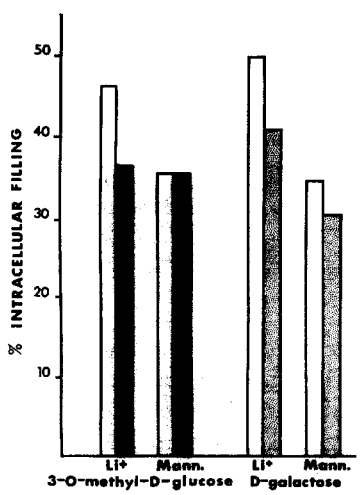

Fig. 2. Mutual inhibition of 3-*O*-methyl-D-glucose and D-galactose. Hamster intestinal segments were preincubated at 37° for 10 min and incubated for an additional 40 min in fresh medium containing sugars and mannitol tracer. The columns are the mean of 6 experiments and represent intracellular filling as a percentage of sugar concentration in the incubation medium. The sugar measured and medium used are designated under the columns. The open columns represent the entry of the test sugar (5 μM) alone and the shaded columns in the presence of the competing sugar (10 mM), which was D-galactose when 3-*O*-methyl-D-glucose was measured and *vice versa*. The effect of the competing sugar is significant in the Li^+ medium in both cases ($P < 0.05$ and $P < 0.025$ for D-galactose and 3-*O*-methyl-D-glucose, respectively). The effects in mannitol medium were not significant.

hibition of entry by phlorizin, in the presence of Li^+, followed the same pattern. Phlorizin, in the presence of mannitol, also inhibited the entry of two actively transported sugars; this may perhaps be related to preliminary observations indicating that sugar movements in a mannitol medium may also be carrier-mediated. The results in Table III are, therefore, consistent with the concept of Li^+ activation of the sugar carrier.

Further evidence for the operation of a Li^+-activated carrier is obtained from Fig. 2. It shows that, in a Li^+ medium, mutual inhibition occurs between galactose and 3-methylglucose, and *vice versa*; the effects in a mannitol medium are not significant.

The effect of Li^+ on the entry of amino acids is of interest, since at least some of them are also actively transported by a Na^+-dependent mechanism[16]. α-Aminoisobutyric acid was chosen as a model substrate; it is not metabolized and its intestinal transport is Na^+-dependent[12,13]. Table IV shows that entry in Li^+ medium is the same as in mannitol medium and is not inhibited by phlorizin. The failure of Li^+ to activate the amino acid carrier in muscle was noted by KIPNIS AND PARRISH[17].

TABLE IV

ENTRY OF α-AMINOISOBUTYRIC ACID IN Li^+ AND MANNITOL MEDIA

Hamster intestinal segments were preincubated at 37° for 10 min and incubated for an additional 40 min in fresh medium containing 0.5 mM amino acid and mannitol tracer. The figures are means of 4 experiments. Deproteinization was done with 5% trichloroacetic acid; other procedures were as with sugars.

Medium	*% filling*
Mannitol medium	32
Li^+ medium	33
Li^+ medium with 0.5 mM phlorizin	34

DISCUSSION

The results of this study will be discussed in terms of the current "carrier" hypothesis of membrane transport[18] and their relevance to the model of intestinal sugar transport proposed by CRANE and co-workers[3,4]. According to this model, the sugar carrier is reversible and symmetrical, with "uphill" sugar movement being a consequence of the asymmetry of carrier activation by monovalent cations. The carrier is assumed to exist in a Na^+-loaded form with high affinity for the sugar and a K^+-loaded form with low affinity. If the asymmetry of ion distribution across the cell membrane is maintained by the Na^+ pump, the distribution of the two forms of the carrier at the two membrane faces will also be asymmetrical: influx of sugar from the region with high Na^+ will be greater than efflux from the intracellular region with high K^+. When the internal concentration of sugar is so high that the low-affinity carrier at the inner face of the membrane has the same degree of saturation as the high-affinity carrier at the outer face, a steady state will be reached. The concentration difference thus established will depend on the degree of asymmetry of ion distribu-

tion and the difference in affinities of the two carrier forms for the particular sugar being transported.

The principal evidence in favour of this model is that when ionic gradients across the cell membrane are reduced or abolished, the asymmetry of sugar fluxes undergoes the same change[2]; and if the Na^+ concentration outside the cell is made lower than inside, the asymmetry of sugar fluxes will be reversed[19,20]. To achieve and maintain such a reversed distribution the Na^+ pump must be completely blocked, and this has been difficult to achieve experimentally. If inhibition of the Na^+ pump is incomplete, there remains some doubt whether the more rapid sugar entry in the presence of Na^+ truly reflects activation of the carrier or whether it is due to some residual activity of the Na^+ pump. This latter explanation would be in agreement with the hypothesis of CSÁKY[5]. Experiments where sugar outflow is measured are not open to this alternative interpretation, but even there uncertainties exist which could be avoided if an ion were available which activates sugar entry but is not a substrate of the Na^+ pump.

Evidence regarding Li^+ interaction with the carrier will be discussed first. As shown in Fig. 1, sugar entry was significantly greater in the presence of Li^+ than of mannitol, choline$^+$ of K^+. Compared to mannitol, a non-ionic substance believed not to influence sugar entry, K^+ is inhibitory, as already reported by others[4]. Choline$^+$ seems to be inert; Tris was also reported not to influence sugar entry[21]. In terms of the model described above, Li^+ can be said to activate the sugar carrier and K^+ to inhibit it. It is, perhaps, significant that only ions of small size (Li^+, Na^+, K^+) interact with the carrier, whereas larger ones, such as choline$^+$ and Tris do not interact. This is consistent with the suggestion of CRANE, FORSTNER AND EICHHOLZ[4] that the attachment of appropriate ions may induce conformational changes in the carrier, making it more or less receptive to sugar.

The curve relating sugar transport rates to graded increases in Na^+ concentration in the medium was shown by BOSAČKOVA AND CRANE[21] to be convex, *i.e.* approaching a saturation value when mannitol or Tris were used to partially replace Na^+, but to have a concave shape when K^+ or Li^+ were used. The latter was interpreted as indicating competition between the ions for attachment to the carrier. In this context our results suggest that Li^+ binding to the carrier causes a small activation of the carrier when it is alone, but antagonizes the activation produced by Na^+ when both ions are present. The action of Li^+ on the carrier would thus be that of a partial agonist, analogous to the action on the acetylcholine receptor of the choline compounds studied by STEVENSON[22].

Interaction of Li^+ with the carrier itself is further supported by experiments with phlorizin, which competitively inhibits[14] sugar transport in the intestine, presumably through interaction with the sugar carrier. Therefore, if an ion activates the sugar carrier, the resulting increased sugar entry should be inhibited by phlorizin, as demonstrated in Li^+ medium. Inhibition by phlorizin is also seen in K^+ medium and, with some sugars, in mannitol medium (Table III), suggesting that sugar entry in these media may occur *via* the low-affinity carrier form. This explanation would also be consistent with preliminary experiments indicating that sugar entry has the same V in Li^+ and mannitol media; earlier[4] a single V was found in Na^+, K^+ and Tris$^+$ media.

The degree of phlorizin inhibition in a mannitol medium varies with different actively transported sugars, as shown in Table III. This could mean that there are con-

siderable variations in the affinity of sugars for the "non-activated" carrier, as well as for the "activated" carrier. Consequently, the degree of activation of the carrier by Na^+ should also vary from sugar to sugar. On this basis it may be understood why ROSENSWEIG, COCCO AND HENDRIX[23] found no difference between the intestinal absorption of D-xylose in Na^+ or Li^+ medium *in vivo*. Entry of this sugar is little activated by Na^+ (ref. 24) and its active transport can be demonstrated only at very low concentrations[25], and the activating effect of Na^+ may not have been detectable under the conditions used.

Other data presented here also suggest Li^+ activation of the carrier. The data in Table III show that the activating effect of Li^+ is limited to sugars normally accepted by the carrier, and Fig. 2 illustrates mutual inhibition of a pair of sugars in Li^+ medium, a phenomenon typical of carrier function.

The results presented here show that the effect of Li^+ on sugar entry may be ascribed to interaction with the sugar carrier. They also provide indirect evidence that interaction with the Na^+ pump is not involved, in agreement with CLARKSON AND ROTHSTEIN[7] who showed that Li^+ is not actively transported in rat intestine. However, a small active transport of Li^+ by the Na^+ pump has been found in some other tissues[26,27]. There are no data on Li^+ transport in hamster intestine. To examine the possible role of Li^+ transport by the Na^+ pump the experiments shown in Table IV were done. If Li^+-activated sugar entry is caused by Li^+ interaction with the Na^+ pump, Li^+ should also activate the entry of amino acids, the transport of which is dependent on the Na^+ pump but mediated by a different carrier[16,28]. The failure of Li^+ to activate the entry of α-aminoisobutyric acid (Table IV), in contrast to its activating effect on sugar entry, suggests that the Li^+ effect is not at the level of the Na^+ pump which is common to both these processes.

The data presented also suggest that the entry of actively transported sugars in the absence of ions may occur *via* "non-activated" carrier, rather than by diffusion. This possibility merits further study.

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