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ACTIVE TRANSPORT OF *MYO*-INOSITOL AND ITS RELATION TO THE SUGAR TRANSPORT SYSTEM IN HAMSTER SMALL INTESTINE

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SUMMARY

Incubation *in vitro* of segments of hamster small intestine has been used to study the mode of absorption of *myo*-inositol. By all current criteria, *myo*-inositol is actively transported. We have observed accumulation against a concentration gradient, energy-dependence, Na^+-dependence, phlorizin sensitivity, and saturation kinetics with a K_m of 0.14 mM. Sugars which interact with and undergo the translocation step of glucose transport are non-competitive inhibitors of the transport of *myo*-inositol. *myo*-Inositol has no effect on the D-glucose transport system. Phlorizin interacts competitively with the *myo*-inositol binding site with an affinity 10–100-fold less than it has for the common sugar binding site. These results indicate that the pathway for *myo*-inositol to cross the brush border membrane is not entirely the same as the D-glucose pathway. However, they also indicate that there is an interaction between the two at the level of translocation.

INTRODUCTION

The uptake of *myo*-inositol against a concentration gradient by kidney cortex slices has been observed[1–4] and characterized[3–4] as Na^+-dependent, energy-dependent and inhibited by phlorizin. These features are similar to those of the uptake of glucose and its closely related analogs (glucose and analogs) by kidney cortex slices[5–6] and preparations of small intestine[7]. Moreover, *myo*-inositol has structural features resembling those of glucose[8]. Consequently, we were interested to study the uptake of *myo*-inositol by the intestine and to find out whether its site and mechanism of uptake are related to those of glucose and analogs. The following is a record of our findings.

METHODS AND MATERIALS

Incubation technique

Experiments were performed by the *in vitro* technique of CRANE AND MANDELSTAM[9] as modified in a more recent publication[10]. Hamsters were fasted overnight.

Abbreviation: PD, potential difference.

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Approx. 200–300 mg wet weight of tissue were placed in 25-ml erlenmeyer flasks containing 10 ml of KREBS–HENSELEIT[11] phosphate buffer with the appropriate added substrates. The buffer was gassed with 100% O_2. In some experiments the buffer was modified in replacing Na^+, to the extent indicated in the graphs, with $Tris^+$. Isoosmolarity was preserved by adding substrates as a 0.3 M solution and an equimolar amount of D-mannitol was added to the controls. In K_m experiments, when the tissue was incubated at different substrate concentrations with a constant concentration of an inhibitor or at a constant substrate concentration with different concentrations of inhibitor, D-mannitol was added to the control in the same concentration as the inhibitor. Tracer amounts of D-[^{14}C]mannitol were added as a marker for extracellular space[12].

Transmural potential difference measurements

The incubation procedure and apparatus for potential difference (PD) measurements were based on the description by LYON AND CRANE[13]. A detailed description of the modified apparatus and the measurement of PD values has been given in a more recent publication[14].

Compounds

The following compounds were from commercial sources: D-mannitol, L-ascorbic acid and D-galactose from Fisher Scientific Corp.; 2-deoxyglucose and phlorizin from Nutritional Biochemicals; 6-deoxy-L-galactose from Pfanstiehl Laboratories, Inc.; 3-*O*-methylglucose from Calbiochem.; L-glucose, β-methylglucoside, *myo*-inositol and Tris from Sigma Chemical Comp.; D-glucose from J. T. Baker Chemical Company; 2,4-dinitrophenol from Matheson, Coleman and Bell; and radioactive *myo*-[2-^{3}H]-inositol from New England Nuclear Corp. The last compound had a specific activity of 3740 mC/mmole with a purity greater than 99% as determined by paper chromatography using the following systems: phenol–water (45:16, w/v), *n*-butanol–ethanol–water (50:32:18, by vol.) and *n*-butanol–acetic acid–water (4:1:5, by vol.).

Although the literature[1] indicates that only the kidney, among mammalian tissues, metabolizes *myo*-inositol to an appreciable extent, we wished to exclude intracellular metabolism of *myo*-inositol as a factor in our results with intestine. Tissue water extracts after the incubation procedure with radioactive *myo*-[2-^{3}H]inositol were subjected to paper chromatography using the following solvent systems: methanol–ethanol–water (45:45:10, by vol.) and phenol–water (45:16, w/v). In both solvent systems only a single component was obtained in the tissue extracts and medium by radioactive scanning with a VANGUARD autoscanner and subsequent counting with a Beckmann liquid scintillation system.

Analytical methods

Incubations were terminated by removal of the tissue from the incubation mixture. Tissue and media were processed for assay as described by CRANE AND MANDELSTAM[9]. *myo*-[2-^{3}H]Inositol, D-[^{14}C]glucose and D-[^{14}C]mannitol were assayed with the Beckman liquid scintillation system.

Calculation of data

Results are expressed either as rate of entry in μmoles of substrate accumulated per ml of tissue water in a given time, assuming a water content of approx. 80%

of the tissue wet weight[9], or as percent filling = 100 × (mmoles/ml tissue water)/(mmoles/ml medium (initial)). All data were corrected for the D-mannitol space. The data presented in tables and graphs are averages of duplicates or triplicates.

RESULTS

Time-course of myo-inositol transport

As active transport of glucose and analogs in the intestine is regarded as Na^+-dependent, phlorizin-sensitive, energy-dependent process of accumulation against a concentration gradient[7], we tested for these characteristics.

Rings of everted hamster small intestine were incubated for periods of time up to 30 min. As seen in Fig. 1, *myo*-inositol accumulated over the entire period finally achieving a concentration gradient of about 3-fold when an initial medium concentration of 0.03 mM was used. The figure shows also the inhibitory effect of β-methyl-D-glucoside.

As can be seen from Table I, the addition of 2,4-dinitrophenol, phlorizin or *N*-ethylmaleimide decreased the uptake of *myo*-inositol markedly. Phloretin was

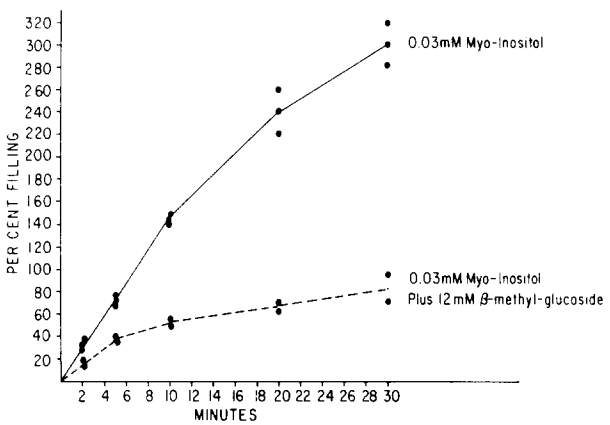

Fig. 1. Uptake of *myo*-[2-^{3}H] inositol by rings of everted hamster small intestine. The initial concentration of *myo*-inositol was 30 μM. Shown also is the effect of β-methylglucoside on the uptake of *myo*-inositol.

TABLE I

ACTIVE TRANSPORT OF *myo*-INOSITOL BY HAMSTER SMALL INTESTINE

Conditions	% *Filling**	% *Inhibition*
Expt. 1 (30 min incubation)		
Control (0.06 mM *myo*-inositol)	306	—
2,4-Dinitrophenol (0.5 mM)	66	78
Expt. 2 (20 min incubation)		
Control (0.03 mM *myo*-inositol)	222	—
Phloretin (0.1 mM)	205	8
Phlorizin (0.1 mM)	80	64
N-Ethylmaleimide (1 mM)	129	42
$Tris^+$ medium (132 mM $Tris^+$, 14 mM Na^+)	64	72

* Average of duplicates.

without substantial effect as compared to phlorizin at the same concentration. Replacement of Na^+ by $Tris^+$ reduced uptake.

Effect of various sugars and glycosides on myo-inositol transport

Tissue was incubated with *myo*-inositol in the presence of several substrates of the pathway of glucose and analogs and several other sugars which appear not to be substrates[7,15]. The results are shown in Table II. Actively transported sugars and glycosides, sharing the pathway of glucose and analogs, namely D-galactose, D-glucose, β-methyl-D-glucoside, arbutin, 3-*O*-methyl-D-glucose, D-xylose and L-glucose inhibited transport of *myo*-inositol. Passively transported compounds, namely, 2-deoxy-D-glucose and D-fructose did not inhibit. Also, 6-deoxy-L-galactose which is a competitive inhibitor of the carrier of glucose and analogs but is not transported[14], was without significant effect. When 30 mM *myo*-inositol was tested as inhibitor of the transport of 1.25 mM D-glucose, no inhibition was seen.

TABLE II

EFFECT OF VARIOUS SUGARS AND GLYCOSIDES ON THE TRANSPORT OF *myo*-INOSITOL AND OF *myo*-INOSITOL ON GLUCOSE TRANSPORT

All incubations were of 10-min duration. Compounds tested for inhibitory capacity were added at a concentration of 30 mM. The values given are averages of duplicates.

Conditions	% *Filling*	% *Inhibition*
Expt. A		
myo-Inositol (0.03 mM)	208	—
plus D-galactose	66	68
arbutin	31	85
D-xylose	131	37
L-glucose	160	23
2-deoxy-D-glucose	193	7
Expt. B		
myo-Inositol (0.03 mM)	250	—
plus β-methylglucoside	42	83
D-glucose	62	75
3-*O*-methyl-glucose	161	35
6-deoxy-L-galactose	252	0
D-fructose	235	6
Expt. C		
D-Glucose (1.25 mM)	1570	—
plus myo-inositol	1505	4

Evaluation of apparent transport K_m and nature of inhibition by D-glucose

Transport of *myo*-inositol exhibited typical saturation kinetics with a K_m on the order of 0.14 mM and inhibition by D-glucose was non-competitive. Because of the importance of this point, the data are plotted by the sensitive methods suggested by HANES[15] ($[S]/v$ *vs.* $[S]$) and by WOOLF[16] (v *vs.* $v/[S]$) and presented in Fig. 2.

In order to determine the K_i of D-glucose, constant concentrations of the substrate *myo*-inositol, were incubated with different concentrations of the inhibitor glucose, for 10 min. The data are plotted by the method of DIXON[17] in Fig. 3 from which an inhibitor constant (K_i) of 7 mM may be derived. In a similar experiment,

not shown, β-methylglucoside (Fig. 1) also acted non-competitively with a K_i of 6 mM. These K_i values are higher than the K_m values of these sugars for the glucose transport system[7, 13].

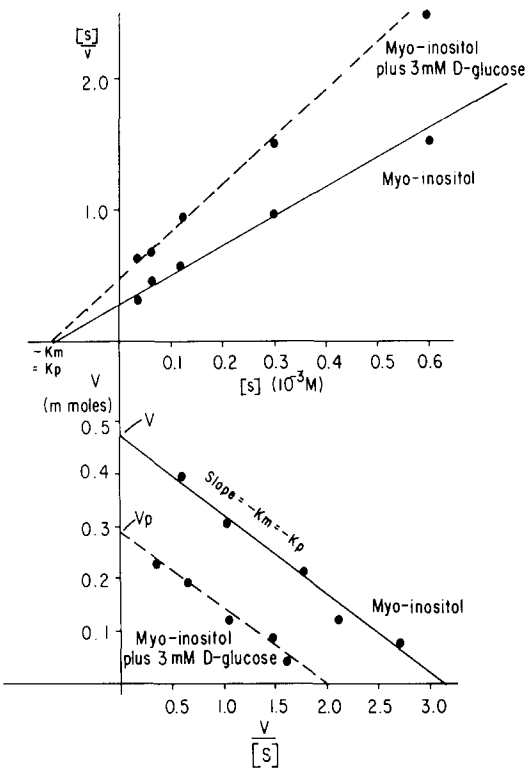

Fig. 2. Effect of D-glucose on the apparent transport K_m of *myo*-inositol. Mean values are plotted by the method of HANES[15] ($[S]/v$ *vs.* $[S]$), upper, and WOOLF[16] (v *vs.* $v/[S]$), lower.

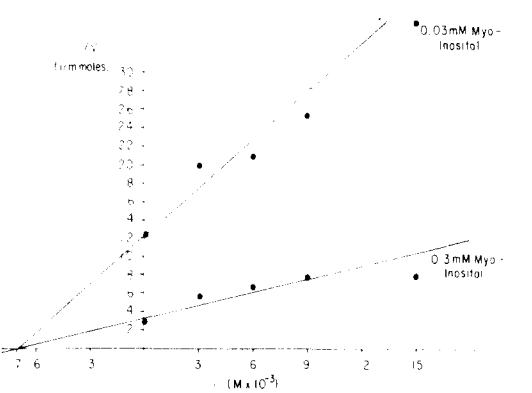

Fig. 3. Determination of the inhibitor constant (K_i) of D-glucose *vs.* uptake of *myo*-[2-^{3}H]inositol by rings of hamster small intestine. Data are plotted according to the method of DIXON[17]. 10 min incubation.

Nature of phlorizin inhibition

The K_i of phlorizin was determined by the method of DIXON[17]. As can be seen from Fig. 4, phlorizin acted as a competitive inhibitor on the transport of *myo*-inositol with a K_i of 80 μM. This value corresponds to an affinity of phlorizin *vs.* *myo*-inositol from 10- to 100-fold less than the affinity of phlorizin for the transport system of glucose and analogs[18].

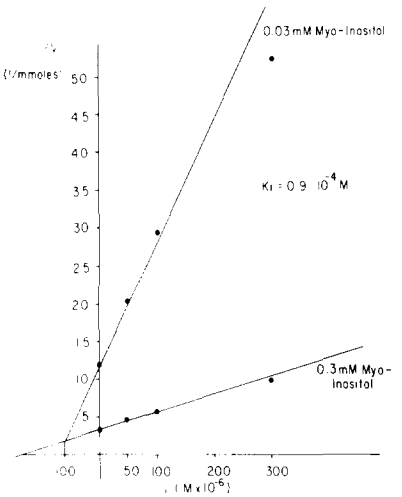

Fig. 4. Determination of the inhibitor constant (K_i) for phlorizin *vs.* transport of *myo*-[2-^{3}H]inositol. The initial concentrations of *myo*-[2-^{3}H]inositol were 0.3 and 0.02 mM. Data are plotted by the method of DIXON[17]. 10 min incubation.

Site of inhibition of various sugars

From Fig. 1 it can be seen that β-methylglucoside inhibits transport of *myo*-inositol at very early time intervals while *myo*-inositol is still transported down its concentration gradient. This result suggests that the inhibiting sugar is interfering with the *myo*-inositol transport system from the external side of the brush border membrane.

In order to test the possibility that inhibitory sugars may exert their action after entering the cell, rings of hamster small intestine were incubated for 10 min with several different sugars; namely, D-glucose, D-galactose and D-fructose. Controls were incubated with equimolar concentrations of D-mannitol. The incubated tissue was recovered, rinsed with fresh buffer and reincubated with *myo*-inositol. The results are presented in Table III. It can be seen that preincubation with D-glucose and D-fructose had no inhibitory effect on subsequent *myo*-inositol transport. The effect of D-galactose, though appreciable, was clearly not of a magnitude observed with D-galactose in the medium (Table II).

Effect on transmural PD

Sugars which enter the intestinal mucosal cell by a Na^+-dependent carrier mechanism may induce a rise in the transmural PD[13,19,20]. The value of the rise of PD at equimolar sugar concentrations is related to the affinity of the sugars for the Na^+-dependent sugar carrier. D-Glucose having the highest affinity for the sugar

TABLE III

EFFECT OF PREINCUBATION WITH VARIOUS SUGARS ON THE SUBSEQUENT TRANSPORT OF *myo*-INOSITOL

Tissues were first incubated for 10 min in 10 ml KREBS–HENSELEIT[11] phosphate buffer containing the indicated compounds at a concentration of 30 mM. Then they were rinsed for about 10 sec in fresh buffer at room temperature, and incubated again for 10 min in 10 ml buffer containing 0.03 mM *myo*-[2-^{3}H] inositol. The values are averages of duplicates.

Sugar in preincubation period	% *Filling*	% *Inhibition*
D-Mannitol	228	—
D-Glucose	204	10
D-Galactose	165	23
D-Fructose	201	12

transport system (1 mM) induces a maximal ΔPD[13], where as L-glucose, $K_m = 65$ mM (ref. 10) when used in the same concentration range induces a very small change in ΔPD[14]. *myo*-Inositol has an affinity for its transport system, $K_m = 0.15$ mM, higher than D-glucose for its transport system, but in comparison to D-glucose and other actively transported compounds using the pathway of glucose and analogs, a very low maximal velocity (see DISCUSSION). As can be seen in Table IV, *myo*-inositol did not induce a rise in ΔPD.

TABLE IV

EFFECT OF *myo*-INOSITOL ON THE TRANSMURAL PD IN HAMSTER INTESTINE

Potential difference values were obtained 30 sec after transfer and are averages of four experiments. They are not corrected for D-mannitol osmotic streaming potential.

Substrate (mM)	*Substrate-induced PD in mV*
D-Glucose	
1	+ 5.5*
5	+ 9.6
10	+ 10.1
myo-Inositol	
1	− 0.1
3	− 0.3
6	− 0.4
12	− 0.8

* Data taken from Ref. 10.

What was observed was only a concentration-dependent negative potential equal in magnitude to that induced by D-mannitol at the same concentrations[14]. Our interpretation of this finding is that the number of carriers available for *myo*-inositol is not enough to translocate Na^+ at the rate required to give a significant positive change in transmural PD in the face of the osmotic effect of the substrate. Similar observations have been made in our laboratory by N. R. STEVENSON (personal communication) with L-ascorbic acid, which has a high affinity for a Na^+-dependent transport system in the guinea pig but a very low v_{max} (ref. 21).

DISCUSSION

The results presented show that *myo*-inositol exhibits all criteria for it to be accepted as an actively transported compound in hamster small intestine. The observations are, accumulation against a concentration gradient, energy-dependence, Na^+-dependence and saturation kinetics with an apparent transport K_m of 0.14 mM.

We tested whether *myo*-inositol might share the pathway of glucose and analogs for its entry into the mucosal cell though *myo*-inositol does not fit the minimal structural requirements for active sugar transport previously recorded[7,15]; *myo*-inositol is a substituted cyclohexane rather than a pyran. The tests were especially interesting, from the point of view of specificity, owing to the recent observation by D. R. CRITCHLEY (personal communication) that the oxygen in the pyranose can be replaced by sulfur, as in 5-thioglucose, with retention of interaction with and transport by the glucose pathway. The ring oxygen of glucose and analogs is thus not a critical structural component. However, our results indicate that *myo*-inositol does not bind to the binding site of glucose and analogs.

As detailed above, we found that transport of *myo*-inositol was inhibited by actively transported sugars with those having a high affinity for the transport of glucose and analogs inhibiting more strongly than those with a low affinity. It was not inhibited by D-fructose and 2-deoxy-D-glucose. In converse experiments, *myo*-inositol did not inhibit transport of glucose (Table II) or even of such a weak substrate as L-glucose (not shown) in spite of the high affinity of *myo*-inositol for transport: 0.14 mM. Kinetic studies of the inhibitions revealed them to be non-competitive.

In a previous paper[14] we have presented evidence for a second step in carrier transport of glucose and analogs in the intestine; a step which occurs after the initial binding interaction between carrier and substrate. The evidence adduced was the fact that 6-deoxy-L-galactose forms a normal complex with the carrier, including associative interaction with Na^+, but is not translocated. In the above experiments, 6-deoxy-L-galactose was without effect on *myo*-inositol transport.

The most revealing comparison in this regard is with L-glucose. As shown previously[14], 6-deoxy-L-galactose, as an inhibitor, had three times the affinity of L-glucose, as a substrate. Since L-glucose inhibits *myo*-inositol transport (Table II), the same could have been expected of 6-deoxy-L-galactose were substrate binding the only event necessary to the inhibition. Consequently, it may be inferred that the effect of transported sugars on *myo*-inositol transport is not a result only of substrate interaction with the binding site of the carrier of glucose and analogs. This, taken together with the non-competitive effect of the transported sugars, permits the conclusion that interference of glucose and analogs with *myo*-inositol transport occurs at the level of the second step, which may be or include translocation.

The failure of *myo*-inositol to influence glucose transport is entirely consistent with this interpretation. Whatever the nature of the second step, its expression is seen in translocation and estimated totally by extrapolation of various rate data to a maximal rate. The extrapolated maximal rate of *myo*-inositol transport is in the range 0.5–5 μmoles/g tissue per h. The extrapolated maximal rate of glucose and galactose transfer by hamster intestine is 100–200 times greater[9]. Assuming a 1:1 relationship between binding and translocation, it is clear that *myo*-inositol could

effect little more than one, at most a few, percent inhibition of transport of glucose and analogs.

The experiments with phlorizin seem, at this point in time, to be somewhat less than revealing. Phlorizin has been repeatedly shown to be a highly potent, competitive inhibitor for the transport system of glucose and analogs[18,23,24]. It also acts as a competitive inhibitor of *myo*-inositol transport (see above). The conclusions that one might draw from this fact are obscured by the knowledge that phlorizin inhibits a variety of enzymes and enzyme systems[15,25,26], which are more related by the carbohydrate nature of their substrates than by involvement in membrane transport reactions.

The special interest of these studies following on those recently reported on the need to formulate a second step[14] is that we now appear to be obtaining kinetic information on that part of the system which lies between the binding interactions at the two sides of the membrane.

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