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A DISTINCT D-FRUCTOSE TRANSPORT SYSTEM IN ISOLATED BRUSH BORDER MEMBRANE

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SUMMARY

The transport of D-fructose in isolated rat intestinal epithelial brush border membranes was studied.

1. The rates of D-fructose entry appeared to be slower than D-glucose but faster than either L-glucose or D-mannitol.

2. D-Fructose uptake, measured after 45 s of incubation, was linear up to and saturable above 200 mM while D-mannitol uptake, with identical incubation conditions, continued to be linear.

3. Isolated membranes were preloaded with unlabeled D-glucose or D-fructose. The uptake of labeled D-fructose was stimulated in the D-fructose-preloaded membranes and unaffected in the D-glucose-preloaded membranes. Conversely, D-glucose transport was enhanced in the D-glucose-preloaded membranes and unaltered in the D-fructose-preloaded membranes.

4. No influence of Na^+ or phlorizin on D-fructose uptake could be demonstrated. In the same experiment D-glucose was stimulated approximately 3-fold by Na^+, and strongly inhibited by phlorizin.

5. None of the sugars tested (D-glucose, D-galactose, L-sorbose or D-galatose), at 100 : 1 test inhibitor to substrate ratio decreased D-fructose transport. Using the same membrane preparation, D-glucose transport was inhibited 75 % by D-galactose.

These results suggest the existence of a distinct transport system for D-fructose, separate from that of D-glucose.

INTRODUCTION

Several reports have been published supporting the existence of an intestina transport system for D-fructose. Nevertheless, D-fructose transport has remained the focal point of considerable controversy. Investigators, using both in vivo and in vitro techniques, have produced widely divergent results. Although not as rapidly absorbed as D-glucose and D-galactose, D-fructose has been shown to be absorbed at a sub-

Abbreviation: HEPES, *N*-2-hydroxyethylpiperazine-*N*-2-ethanesulfonic acid.

stantially faster rate than sugars which are thought to traverse the intestine via a passive diffusive mechanism [1–3]. Differences in demonstrating saturability, in inhibition observed with other sugars and phlorizin, and differing Na^+ sensitivity are among the conflicting observations reported [3–11]. A complicating factor is the differences between species in the metabolism of fructose. In guinea pig [12, 13], hamster [14] and dog [15], fructose is reported to be converted to glucose and lactate during absorption. In man [16] and in the rat [17], fructose appears to be absorbed unchanged.

We have recently demonstrated an intact glucose transport system in the highly purified brush border membranes from the epithelial cells of rat small intestine [18]. Glucose transport into the vesiculated membranes has the same characteristics as reported for intact tissue preparations: stereoisomeric differentiation, Na^+ stimulation, phlorizin and D-galactose inhibition.

In view of the many conflicting reports concerning D-fructose transport and the dietary importance of this sugar we decided to investigate its transport using isolated brush border membranes.

MATERIAL AND METHODS

Using Sprague–Dawley rats (200–250 g), brush border membranes were isolated by the method of Hopfer et al. [18] with the modification that D-mannitol and D-sorbitol were used, in some instances, at a concentration of 500 mM.

Uptake procedure

The isolated membranes were incubated at 25 °C in a mannitol–Tris–*N*-2-hydroxyethylpiperazine-*N*-2-ethanesulfonic acid (HEPES) buffer (HEPES buffer adjusted with Tris–hydroxide to pH 7.5). The incubation medium contained, in all cases, Tris-HEPES (1 mM) pH 7.5 and $MgSO_4$ (0.1 mM). Varying incubation concentrations of polyols, sugars and salts are given in the legends. D-Fructose was present in the incubation medium as D-[1-^{3}H] fructose in concentrations ranging from 1–500 mM. In some experiments either D-[1-^{14}C]mannitol or L-[1-^{14}C]glucose were additionally present as control for passive diffusion. All labeled substances were purchased from New England Nuclear (Boston, Mass.). For studying D-mannitol uptake a D-sorbitol buffer was used for the membrane preparation and the incubation medium. No difference in D-fructose, D-glucose or L-glucose uptake was discernible between the mannitol and sorbitol buffers. The uptake of D-fructose was terminated with the removal of an aliquot (20–80 μg protein) from the incubation medium, rapid 50-fold dilution with ice-cold buffer containing 200 mM NaCl, 100 mM mannitol, 10 mM Tris–HEPES pH 7.5, 10 mM $MgSO_4$ and 0.4 μCi/ml D-[1-^{14}C]mannitol, when a single isotope was used in the incubation medium. The dilution medium was immediately filtered through a Sartorius filter (No. 11305, 0.6 μm) and the collected membranes were rinsed once with 4 ml of the dilution buffer without the isotope. The isotope in the dilution medium was used as a correction for unspecific sugar retention by the filter, due to insufficient washing. No binding or retention of fructose alone by the filter was found.

Single experiments are shown. However, all experiments were repeated at least

four times with similar results. Uptake values from duplicate samples of the same membrane preparation were within 8 % of the mean. For control experiments, membranes from the same batch were utilized except in preloading studies where aliquots of brush borders from the same suspension were used. An incubation time of 45 s was taken as a convenient time point to approximate rates of uptake and applies to all tables and figures except where indicated otherwise. Appropriate correction factors were employed for changes in vesicular volume due to increasing osmolarity (e.g. the uptake of D-fructose was multiplied by two when the membranes were prepared in a 500 mM D-mannitol buffer and then the uptake was measured in a medium of 500 mM D-mannitol plus 500 mM D-fructose).

Analytical procedures

The filters were dissolved in a fluor consisting of toluene–Triton X-100 (2 : 1, v/v) containing 8 g 2-(4′-*tert.* butylphenyl)-5-(4″-biphenyl)-1,3,4-oxdiazole (Ciba-Geigy) per l toluene and counted in a Nuclear Chicago (Mark I) liquid scintillation counter. Appropriate corrections for counting efficiency, background and quenching were applied. Protein determination was carried out by the method of Resch et al. [19] as previously reported [18].

Chemical identification of D-fructose was performed by thin-layer chromatography. Samples of membranes (collected on the filters) containing transported D-fructose were extracted with 30 % ethanol. The extracted D-fructose as well as the labeled D-fructose employed in the incubation medium were chromatographed in two solvent systems: 1-butanol–acetic acid–diethyl ether–water (9 : 6 : 3 : 1, by vol.) and 2-propanol–ethyl acetate–water (7 : 1 : 2, by vol.). Reference compounds were sprayed with benzidine–trichloroacetic acid [20] and aniline–diphenylamine [21]. The chromatogram was then separated into 1-cm strips from the origin to the solvent front. These strips were eluted with 30 % ethanol the eluate concentrated and counted in a liquid scintillation vial. All of the isotope taken up by the brush border membranes could be quantitatively identified as D-fructose; in particular no conversion to D-glucose or fructose esters could be detected.

RESULTS

Time course

When isolated membranes were incubated simultaneously with D-fructose and D- or L-glucose, or D-mannitol, a differential time course of uptake was obtained (Fig. 1). The kinetics of D-fructose uptake were faster than those of L-glucose or D-mannitol but not as rapid as D-glucose. It has been previously shown that D- and L-glucose and D-mannitol do not bind but rather enter into an osmotically active space, the brush border membrane vesicle [18]. D-Fructose reaching the same equilibrium value as D- and L-glucose indicated that it is also entering this same vesicular space. The explanation for the unusual shape of the D-glucose uptake curve, occurring in the presence of a NaSCN gradient, has been given previously [22].

Additionally, when membranes, preloaded with labeled D-fructose, were diluted into a medium without the sugar D-fructose was released indicating that D-fructose uptake was reversible.

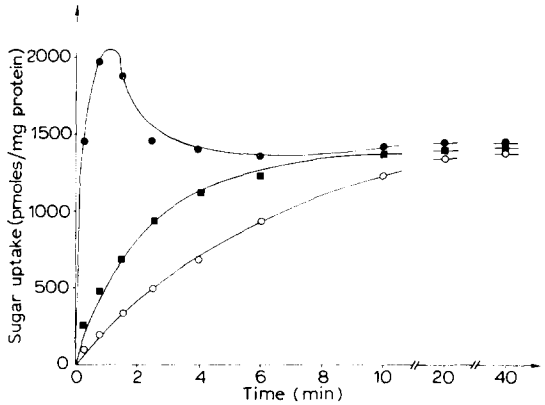

Fig. 1. Time course of D-fructose and D- and L-glucose uptake. Incubation medium contained (final concn): D-[1-^{3}H]fructose (1 mM; ■), D-[1-^{3}H]glucose (1 mM; ●) or L-[1-^{14}C]glucose (1 mM; ○), D-mannitol (100 mM) and NaSCN (100 mM).

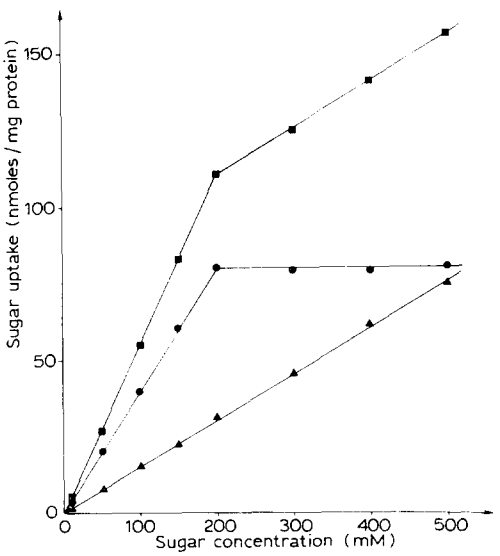

Fig. 2. Saturation of D-fructose transport. Uptake of D-fructose (■) D-mannitol (▲) and ΔD-fructose (●), defined as D-fructose minus D-mannitol. Composition of incubation medium: D-[1-^{3}H]-fructose and D-mannitol (500 mM) or D-[1-^{3}H]mannitol and D-sorbitol (500 mM).

Saturation

D-Fructose transport was linear with respect to concentration up to 200 mM. At concentrations above 200 mM D-fructose uptake deviated from linearity (Fig. 2). D-Mannitol, employed at the same concentrations failed to show a deviation from linearity. When D-fructose transport was corrected for diffusive entry by subtraction of D-mannitol uptake at the same concentrations, the Δ (D-fructose uptake) remained constant from 200–500 mM.

Counter transport

Membrane fractions were preloaded by being prepared in two different buffers: 300 mM D-mannitol–1 mM Tris–HEPES (pH 7.5)–0.1 mM $MgSO_4$, plus (a) 200 mM D-fructose or (b) 200 mM D-glucose. As control, a sample of membranes from the same preparation were prepared in 500 mM D-mannitol–1 mM Tris–HEPES (pH 7.5)–0.1 mM $MgSO_4$. Fig. 3 shows the effects of the preloaded membranes on D-fructose and D-glucose transport. D-Fructose uptake was noticeably stimulated in the D-fructose-preloaded membranes (Fig. 3a), whereas it was unchanged (with respect to control levels) by preloading the membranes with D-glucose. Conversely, D-glucose uptake was markedly stimulated in the D-glucose-preloaded membranes (Fig. 3b) and unaltered by the preloading with D-fructose.

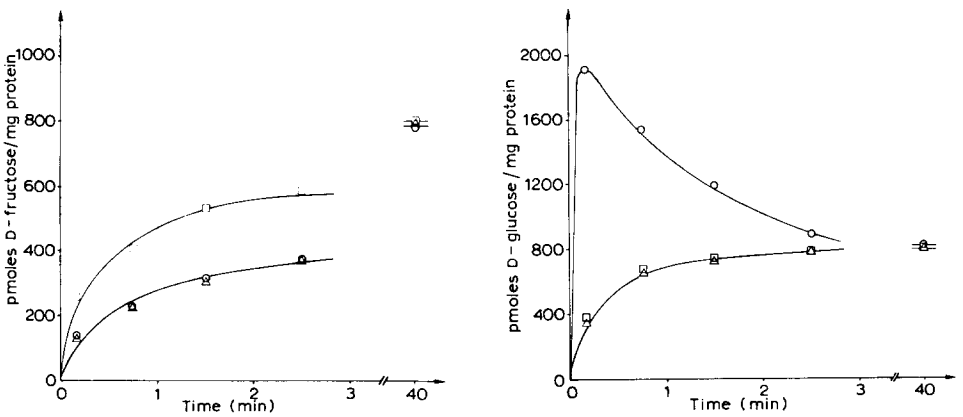

Fig. 3. Effect of preloading on D-fructose and D-glucose entry. Membranes were preloaded with sugars as described in Results. D-Fructose-preloaded membranes (□); D-glucose-preloaded membranes (○); D-mannitol-preloaded membranes (△). Incubation medium contained D-mannitol (500 mM), NaSCN (100 mM) and 1 mM of either D-[1-^{3}H]fructose (a) or D-[1-^{3}H]glucose (b).

TABLE I

INFLUENCE OF VARIOUS ELECTROLYTES ON D-FRUCTOSE AND D-GLUCOSE UPTAKE

Incubation medium composition (final concn): D-[1-^{3}H]fructose (1 mM) or D-[1-^{3}H]glucose (1 mM), D-mannitol (100 mM) plus the indicated additions.

Additions (mM)		Uptake of D-fructose (pmoles/mg protein)	Uptake of D-glucose (pmoles/mg protein)
D-Mannitol	(200)	436	475
NaCl	(100)	400	1260
KCl	(100)	405	462
NaSCN	(100)	431	1460
KSCN	(100)	425	500
NaSCN+ phlorizin	(100) (1)	408	200

Influence of electrolytes and phlorizin

The influence of various electrolytes and phlorizin on D-fructose transport is shown in Table I. For comparison, D-glucose uptake values under the same conditions are also shown. D-Fructose transport was neither stimulated by Na^+ nor inhibited by phlorizin, whereas D-glucose was stimulated approximately 3-fold by Na^+ and strongly inhibited by phlorizin.

Influence of other sugars

The effect of various sugars in the incubation medium on D-fructose transport is demonstrated in Table II. D-Glucose uptake under identical conditions is again shown for comparison. At an inhibitor to substrate ratio of 100 : 1, no significant inhibition of D-fructose transport by any of the sugars tested occurred. D-Glucose uptake was inhibited 75 % by D-galactose but unaffected by the other sugars. Uptake in 100 mM mannitol was used as reference. Employing higher concentrations of the test inhibitor sugars (200 mM) on D-fructose transport gave similar results as those presented.

TABLE II

INFLUENCE OF VARIOUS SUGARS ON D-FRUCTOSE AND D-GLUCOSE TRANSPORT

Incubation medium contained (final concn): D-[1-^{3}H]fructose (1 mM) or D-[1-^{3}H]glucose (1 mM), D-mannitol (100 mM), NaSCN (100 mM) and test inhibitor (100 mM). Percentage of inhibition indicated in parentheses.

Test inhibitor (100 mM)	Uptake of D-fructose (pmoles/mg protein)	Uptake of D-glucose (pmoles/mg protein)
D-mannitol	353 (0)	1485 (0)
D-glucose	335 (5)	
D-galactose	346 (2)	371 (75)
D-fructose		1410 (5)
L-sorbose	350 (1)	1484 (0)
D-tagatose	349 (1)	1471 (0)

DISCUSSION

The isolated membrane preparation employed has several advantages over conventional tissue methods. Binding can be differentiated from transport. A finer degree of control over experimental conditions is possible, due to the simplified system. Extraneous effects such as tissue swelling which may have a marked effect on non-electrolyte absorption are eliminated with this type of preparation. Furthermore, no metabolism of D-fructose by the membrane preparation occurred. All the isotope that was transported could be quantitatively identified as D-fructose.

Several observations support the concept that the measured D-fructose uptake represents carrier-mediated translocation across the microvillus membrane: (1) D-fructose was taken up to the same extent as D- and L-glucose, known to enter an osmotically active space [18]; (2) the uptake was reversible; (3) the rate of uptake, measured after 45 s of incubation, showed saturation above 200 mM D-fructose; and (4) D-fructose uptake was enhanced by preloading with this hexose. Fructose

transport in microvillus membranes was insensitive to Na^+ in the incubation medium. KCl and KSCN, likewise, did not exert any significant influence. With membranes from the same preparation, D-glucose transport was stimulated 3-fold by NaSCN and more than 2-fold by NaCl. With 1 mM phlorizin D-glucose transport was very strongly inhibited while, again, D-fructose transport remained unaffected.

Schultz and Strecker [7] using sheets of rabbit ileum, have reported similar observations. They noted that fructose transport in a Na^+-free medium (replaced by choline chloride) did not significantly differ from control values. D-Fructose transport was unaffected by 0.1 mM phlorizin, Macrae and Neudörffer [6], working with rat small intestine segments, found that fructose absorption showed a moderate decrease when Na^+ was replaced with K^+, however, when NaCl was replaced with choline chloride a significant increase in fructose absorption occurred. Tris–HCl caused a marked reduction in fructose uptake. Because of the varying effects of the replacement ions, the authors concluded there was not a strict Na^+ dependence for fructose transport. In addition, 0.1 mM phlorizin failed to inhibit fructose transport. Similarly, Honegger and Semenza [11] noted that fructose uptake in hamster small intestine was not inhibited by the absence of Na^+ or the presence of 1.2 mM phlorizin. In contrast, Gracey et al. [8] reported that when Na^+ was replaced with K^+, Li^+ or $Tris^+$, the uptake of D-fructose was markedly reduced and accumulation against a concentration gradient could no longer be demonstrated. Phlorizin, at concentrations ranging from 0.5–5.0 mM, partially inhibited D-fructose transport.

None of the sugars employed in our experiments were successful in inhibiting D-fructose transport. It is interesting that L-sorbose and D-tagatose, both stereoisomers of D-fructose, had no inhibitory power. D-fructose in turn, was unable to inhibit D-glucose or D-galactose entry. This is in contrast to a 10% decrease in absorption of D-fructose in the presence of L-sorbose as noted by both Macrae and Neudörffer [6] and Schultz and Strecker [7]. Gracey et al. [8], likewise, reported moderate inhibition by L-sorbose. Finally, Honegger and Semenza [11] reported that L-sorbose was unable to significantly inhibit fructose transport.

The results presented in this paper constitute further support for the concept that D-fructose transport in rat small intestine occurs by a saturable, rate-limiting process. The data are compatible with a carrier-mediated mechanism. The following observations: absence of Na^+ dependence, lack of phlorizin inhibition, lack of inhibition by D-glucose and D-galactose, the inability of D-fructose to inhibit D-glucose entry and finally the failure of D-fructose and D-glucose to serve as mutual counter transport substrates, all point to a fructose transport system separate from that of D-glucose.

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