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On the Multiplicity of Sugar Transport Systems in Guinea Pig Jejunum*

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A study has been made in everted sacs of guinea pig jejunum to see if the two transport systems of glucose analogues characterized at the brush border membrane vesicles are operative. The transport kinetics of D-galactose and α-methylglucoside up to 80 mM concentrations has been studied, as well as the mutual inhibitions between them at low and high concentrations of the substrate and at different concentrations of the inhibitor. Low temperature (20 °C) inhibits galactose transport at 0.1 mM (70 %) and 40 mM (78 %) concentrations. A mass transfer coefficient, KD, somewhat higher for galactose than for α-methylglucoside, was obtained when the transport component was abolished by phlorizin. The transport of D-galactose and α-methylglucoside seemed to be compatible with the function of one system shared by both substrates, which presents greater affinity for α-methylglucoside. The functional existence of two systems of active transport at the brush border of guinea pig was not evidenced in intestinal preparations of whole tissue, due perhaps to the effect of the unstirred water layers. However, differences in KD values and some results of the mutual inhibitions may suggest a second system.

Key words: Intestinal transport, Galactose, α-Methylglucoside, Temperature, Guinea pig, Everted sacs.

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Previous work (7) was carried out to see whether with *in vivo* and *in vitro* techniques, which maintained the intestinal epithelium intact, the existence of two transport systems for glucose and other sugars, recently described by BROT-LAROCHE and ÁLVARADO (1-3) in brush

border membrane vesicles (BBMV) of guinea pig jejunum, could be extended to rat small intestine. Although these preparations with intact tissue give rise to the formation of unstirred water layers (UWL) which hinder the kinetic study of transport (8, 10-12), the experiments showed that sugar entry into the tissue from intestinal lumen could be explained by the concurrence of a non saturable, phlorizin insensitive, passive process and another one of saturable, phlorizin sensitive, mediated transport which, between 0.1 and 80 mM substrate concentrations, acceptably followed Michaelis kinetics for one transport system. Mutual inhibitions between galactose and α-methylglucoside transport and the effects of cytochalasin B and low temperature (20 °C), could be explained under the assumption of one transport system shared by both substrates. These differences can be attributed to species specificity or to distorting effects due to the UWL resistance.

It seemed interesting to undertake the present work on sugar transport in everted jejunum sacs of guinea pig, to find out whether in the same animal species in which the existence of two systems were reported and their properties established (1-3), it was possible to obtain in preparations with intact intestinal epithelium instead of with BBMV, results that would support the functional multiplicity of the transport systems. The experimental data are not conclusive in that respect.

Materials and Methods

Male Dukin Hartley guinea pigs, weighing 260 to 320 g, kept in GLP conditions and fed a mixed diet, were used. After a 24 h fast the animals were sacrificed, and everted jejunum sacs of about 3-3.5 cm in length, according to the WILSON and WISEMAN (13) technique, were prepared. The sacs remainded closed, but empty, and were incubated in 5 ml of mucosal medium, pH 7.4 Krebs-Ringer-Tris (6) at 37 °C with constant stirring and O_2 bubbling for 4 min. At the end of the incubation the sugar entry into the tissue, previous digestion in 0.1 N NO_3H, was measured by radioactivity.

The products and other aspects of the procedure used as well as the statistics analysis are as indicated in a previous work (7).

Results

Kinetics of D-galactose and α-methylglucoside uptake by the tissue. Substrate entry into the tissue from 0.1 to 80 mM concentrations in the absence or presence of 0.5 mM phlorizin in the mucosal medium has been studied. Results (fig. 1) show that the uptake of both substrates is the sum of a passive linear, phlorizin insensitive component and of another one saturable and phlorizin sensitive. The course of transport as a function of the substrate concentration corresponds to Michaelis-Menten kinetics for one system, so that the Lineaweaver-Burk plot offers good reliability ($r = 0.99$) and makes possible to deduce the apparent Km and Vm values which for galactose and α-methylglucoside were 2.86 and 2.27 $\mu mol \cdot g^{-1}$ w.w. $\cdot$ 4 min^{-1} (Vm) and 9.1 and 5 mM (Km) respectively.

The mass transfer coefficient (KD) for the diffusion component was 0.072 $\mu mol \cdot g^{-1}$ w.w. $\cdot$ 4 $min^{-1} \cdot mM^{-1}$ for galactose and 0.05 for α-methylglucoside.

Mutual transport inhibitions between galactose and α-methylglucoside. — As it had been done in rat, the inhibition exerted by 25 mM α-methylglucoside on the transport of 0.1 or 40 mM galactose and that exerted by 25 mM galactose on the transport of 0.1 or 40 mM α-methylglucoside, was measured. In other experiments the concentration substrate of the that acted as inhibitor was kept eight times

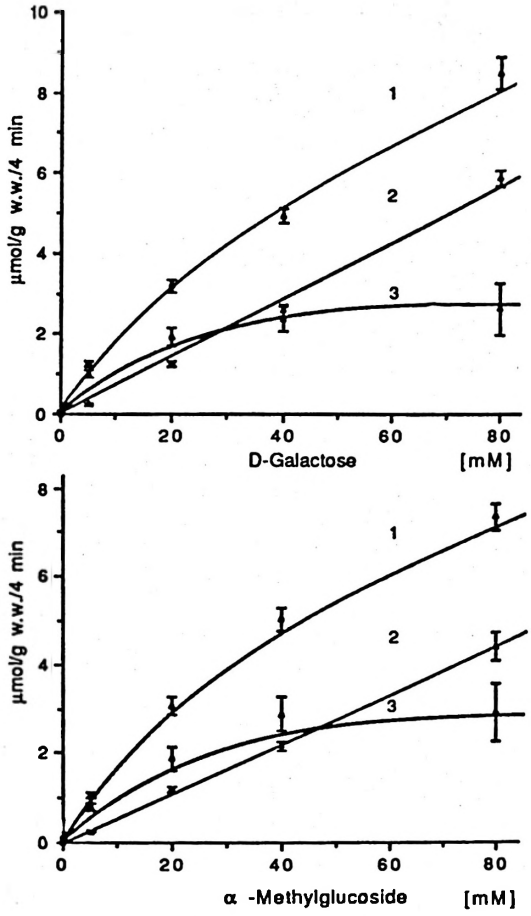

Fig. 1. *Intestinal absorption kinetics of D-galactose and α-methylglucoside in guinea pig jejunum* in vitro.
1) Total absorption; 2) passive diffusion; 3) active transport. Closed and empty everted jejunum sacs. The active transport curve was determined from the difference between total absorption and diffusion. Each point is the mean from 6 to 33 experimental data.

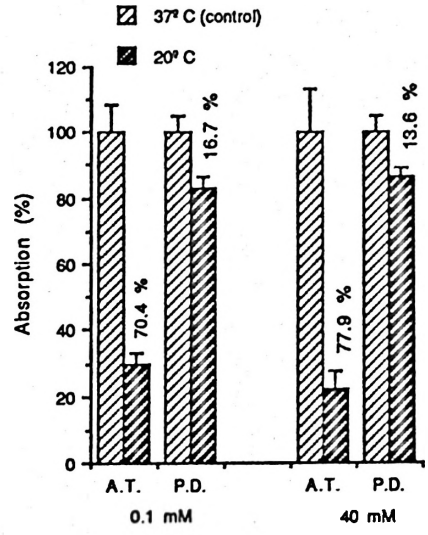

Fig. 2. *Inhibitory effect of low temperature (20 °C) on the active transport and the passive diffusion of 0.1 and 40 mM galactose in guinea pig jejunum* in vitro.
For each series, the absorption in control conditions (µmol · g^{-1} w.w. · 4 min^{-1}) is taken as 100. Inhibitions in %. n = 20-33 (A.T., active transport; P.D. passive diffusion).

higher than that of the substrate whose transport was being measured, the latter being 0.1 or 10 mM.

Table I summarizes the experimental results together with those calculated in the hypothesis of competitive inhibition for just one transport system, whose Vm and Km would be those obtained in the previous section. As it may be seen, there is an acceptable concordance. In every case, the transport values were determined as the difference between the substrate uptake in the absence and in the presence of 0.5 mM phlorizin.

Effect of low temperature. — Galactose entry into the tissue at 0.1 and 40 mM concentrations clearly decreases when the incubation is carried out at 20 °C instead of at 37 °C (fig. 2), which is due to inhibition of the transport component, as the passive component is not significantly affected. The transport inhibition in % is very similar independently of whether the sugar concentration is 0.1 mM (70.3 %) or 40 mM (77.9 %).

Table I. *Mutual inhibitions on the active transport between galactose and α-methylglucoside in guinea pig jejunum* in vitro.

The experimental values and those estimated for the competitiviness for one transport system are included. Units: μmol · g $^{-1}$ w.w. · 4 min $^{-1}$. (Gal, galactose; MG, α-methylglucoside; Vc, transport rate in control conditions; Vi, transport rate in the presence of the inhibitor).

		Experimentals		Calculated	
[mM]		Transport	% Inhibition	Transport	% Inhibition
Gal + MG					
0.1	25	Vc = 0.027 ± 0.0023		0.031	
		Vi = 0.002 ± 0.0009	92.5	0.005	83.9
40	25	Vc = 2.35 ± 0.31		2.33	
		Vi = 1.60 ± 0.37	31.9	1.21	48.1
MG + Gal					
0.1	25	Vc = 0.066 ± 0.0012		0.0045	
		Vi = 0.014 ± 0.0022	78.7	0.0012	73.3
40	25	Vc = 2.87 ± 0.38		2.02	
		Vi = 2.31 ± 0.43	19.5	1.55	23.3
Gal + MG					
0.1	0.8	Vc = 0.025 ± 0.003		0.031	
		Vi = 0.019 ± 0.002	24.0	0.027	12.9
10	80	Vc = 1.60 ± 0.05		1.49	
		Vi = 0.18 ± 0.02	88.8	0.17	88.6
MG + Gal					
0.1	0.8	Vc = 0.062 ± 0.003		0.045	
		Vi = 0.051 ± 0.002	17.7	0.041	8.9
10	80	Vc = 1.84 ± 0.06		1.51	
		Vi = 0.67 ± 0.08	60.3	0.38	74.8

Discussion

As it happened in rat jejunum *in vivo* (7), the uptake of galactose and α-methylglucoside by the tissue with guinea pig jejunum sacs incubated *in vitro* may be explained as the result of a passive entry of a diffusional type, to which a process of a saturable, phlorizin sensitive, mediated transport is added. In the adopted experimental conditions, the KD of the galactose diffusional process is somewhat greater than that of α-methylglucoside. On the assumption that there is just one transport system shared by both substrates, the Vm were very similar although somewhat higher for galactose than for α-methylglucoside, whereas the galactose Km was clearly higher. Resembling results had been reported by others (9) with similar preparations. These results do not demand by themselves the existence of two transport systems, SI and SII, although the fact that the KD for the galactose passive component seems higher than that of α-methylglucoside could indicate the functioning of a SII system masked as a part of the passive component.

The mutual inhibition between the transport of D-galactose and α-methyl-

glucoside at different substrate concentrations agree well with what could be expected assuming competitive inhibition for just one transport system. The comparison between the experimental data and those calculated under that assumption shows enough parallelism. In the hypothesis that two transport systems, SI and SII, the SII being of low affinity and high capacity and not shared by α-methylglucoside (1), were operative, deviations between the experimental data and those calculated for just one system should be expected, especially with high substrate concentrations: the transport of 40 mM galactose, which in good measure would be done with the SII system, would have to be inhibited by 25 mM α-methylglucoside less than what was estimated, whereas the inhibition of 40 mM α-methylglucoside transport, which does not use the SII system, by 25 mM galactose ought to be coincident. Consideration of table I suggests that this seems to occur.

The fact that at 20 °C temperature transport is inhibited in similar proportion when the galactose concentration in the medium is 0.1 or when it is 40 mM, does not make it possible to distinguish the existence of a low affinity system (SII), unoperative at a low temperature. As in these preparations the influence of temperature on many cellular processes is quite great and it bears on the sugar active transport, it could happen that the suggested different termosensitivity of the SI and SII systems was masked.

The results with intact tissue, here presented, only lend a somewhat weak support to the existence of the two systems proposed from experiments with guinea pig BBMV. This may be due mainly to the different experimental conditions, specially to the fact that the effect of the UWL in our preparations may render the function of the SII system less relevant. The presence of the UWL, which reduce the effective substrate concentration at the membrane, would reduce the use of SII system to a proportion lower than what would happen in the absence of the UWL.

It has been reported recently (4, 5) that the sugar physiological concentrations at the lumen of the small intestine are normally between 0.2 and 48 mM, remarkably lower than those that were being admitted. As the UWL have a strong relevance on intact intestine, those concentrations will be still smaller at the luminal membrane, and so it is very doubtful that a system of such a low affinity as the SII system may have physiological signification.

Resumen

Se trata de comprobar en sacos evertidos de yeyuno de cobaya si son operativos los dos sistemas de transporte activo de análogos de glucosa caracterizados en vesículas de membrana luminal de enterocitos. Se estudia la cinética de transporte de D-galactosa y α-metilglucósido hasta concentraciones 80 mM, así como las inhibiciones recíprocas a concentraciones bajas y altas del azúcar sustrato y a distintas concentraciones del inhibidor. También se observa la inhibición por baja temperatura (20 °C) sobre el transporte de galactosa 0,1 mM (70 %) y 40 mM (78 %). La constante de transferencia de masas, KD, obtenida para la galactosa es algo mayor que la del α-metilglucósido. El transporte de D-galactosa y α-metilglucósido parece ser compatible con la función de un único sistema compartido por ambos azúcares que presenta mayor afinidad por el α-metilglucósido. La existencia funcional de dos sistemas de transporte activo en el borde en cepillo de yeyuno de cobaya no se pone de manifiesto en preparados intestinales de tejido completo, quizá por efecto de las capas de agua no agitadas. Sin embargo, las diferencias en los valores de KD y algunos resultados de inhibición mutua podrían sugerir un segundo sistema.

Palabras clave: Transporte intestinal, Sacos evertidos, Galactosa, α-Metilglucósido, Temperatura, Cobaya.

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