REVISTA ESPAÑOLA DE FISIOLOGIA, 47 (4), 209-216, 1991

On the Multiplicity of Glucose Analogues Transport Systems in Rat Intestine*

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(Received on July 7, 1991)

M. P. LOSTAO, A. BERJON, A. BARBER and F. PONZ. *On the Multiplicity of Glucose Analogues Transport Systems in Rat Intestine.* Rev. esp. Fisiol., 47 (4), 209-216, 1991.

A study has been made to test if in intact epithelium of rat jejunum with *in vivo* and *in vitro* techniques, two transport systems for glucose and analogues, as those characterized in brush border membrane vesicles from guinea pig jejunum, are operative. The passive and mediated transport components of the D-galactose and methyl α-D-glucopyranoside intestinal absorption and the mutual inhibitions between both substrates at different relative concentrations have been measured. The effects of cytochalasin B and low temperature (20 °C) on the transport *in vitro* have also been observed. Cytochalasin B inhibits galactose and α-methylglucoside transport at 0.1 and 40 mM concentrations in similar percentage. Transport of 0.1 and 40 mM galactose is inhibited 61 and 77 % respectively by low temperature (20 °C). The transport of galactose and α-methylglucoside could be explained by the assumption of just one transport system shared by both substrates, with a higher affinity for α-methylglucoside. Operation of two systems was not demanded by the results, due perhaps to species specificity or to the distorting action of the unstirred water layers.

Key words: Transport systems, Galactose, α-Methylglucoside, Rat jejunum, Cytochalasin B.

The intestinal absorption of glucose and analogues includes a preferably paracellular passive component (9) and another transcellular active one of cotransport with Na^+ at the enterocyte luminal membrane. Various works on mammal small intestine suggested that cotransport may use more than one system (4-7, 11, 12, 19). More recently the existence of two cotransport systems, one of high affinity and low capacity and another one of low affinity and high capacity, has been postulated at the brush border membrane ve-

* This work was in part supported by a Grant of the "Comisión Interministerial de Ciencia y Tecnología" (PB 86-0407) (Spain).

** M. P. Lostao has a scholarship from the "Programa Nacional de Formación de Personal Investigador" (Spanish Government).

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sicles (BBMV) of rabbit and steer (14, 15), cat (31), guinea pig (1-3) and humans (10, 18). For other authors there might be three systems for aldohexoxe transport in rabbit jejunum or just one with changing properties during the enterocyte maturation (24).

Papers of the ALVARADO group (1-3) in BBMV of guinea pig jejunum distinguish two cotransport systems for glucose and other sugars: System I of high affinity (Km = 0.4 mM) and low capacity (Vmax = 336 pmol · g^{-1} prot. · s^{-1}), active at 35 °C, cytochalasin B insensitive, shared by methyl α-D-glucopyranoside, identifiable with the classic system for Na^+-glucose cotransport, and System II, of low affinity (Km = 24 mM) and high capacity (Vmax = 2,233 pmol · g^{-1} prot. · s^{-1}), inactive when the temperature drops to 25 °C, cytochalasin B sensitive and not shared by α-methylglucoside.

The present work in rat jejunum with intact epithelium and with *in vivo* and *in vitro* techniques, which are less appropriate for kinetic studies than BBMV but much closer to physiological conditions, tries to support the existence of two transport systems similar to those described for guinea pig. The results, however, do not demand the functioning of two transport systems.

Materials and Methods

Male Wistar rats, weighing 150-200 g, fed a mixed diet, kept according to the GLP norms, supplied by the Research Centre of Applied Pharmacology of the University and subjected to a 24 h fast prior to the experiment, have been used.

For *in vivo* absorption the successive absorption technique of PONZ *et al.* (23) has been used, with *in situ* perfusion of a 20 cm long jejunum segment, under anesthesia with urethane (125 mg/100 g). The perfusion solution (10 ml) was 0.9 % NaCl adjusted to 7.4 pH with Tris/HCl, which was recycled at a 6.2 ml/min rate during 4 min long absorption periods. The absorbed sugar was measured as the difference between the initial and final content in that solution. Although the initial and final volume of the perfusion solution was determined by weight, the differences were inferior to 0.8 %, being considered, thereby, equal (10 ml).

For the *in vitro* experiments jejunum everted sacs (30) were used, of about 3-3.5 cm in length, suspended in 5 ml of mucosal medium, 7.4 pH, Krebs-Ringer-Tris (27, phosphate replaced by Tris) at 37 °C with O_2 bubbling and stirring. The incubation periods of the closed, empty sacs in the medium with sugar were of 4 min duration. Along this time, the rate of sugar uptake by the tissue was kept practically constant (data not shown). After the incubation, the sacs were digested in 0.1 M NO_3H to evaluate the sugar entry into the tissue from the mucosal side.

D-galactose was supplied by Merck and methyl α-D-glucopyranoside, 2-deoxyglucose, phlorizin and cytochalasin B by Sigma. The labelled substrates D-(1−^{14}C)-galactose, methyl α-D-(1−^{14}C)-glucopyranoside and 2-(1−^{14}C)-deoxyglucose were supplied by Dupont.

Sugar determination was made by sample radioactivity measurement (in triplicate) in a liquid scintillating counter (Beckman, LS 1800).

Statistic significance was determined by means of the Student's t test for non paired data (20) and the linear regression straight lines were calculated by the minimum squares method (29) with the aid of the Macintosh Statview program.

Results

In vivo *absorption kinetics of D-galactose, α-methylglucoside and 2-deoxyglucose.* — The three sugars undergo very slight or no metabolization at the intestinal wall. The absorption of the substrates

has been measured between 0.1 and 80 mM concentrations. For those higher than 40 mM, the NaCl concentration was lowered to avoid hypertonicity, which does not appreciably affect absorption (21).

Figure 1 shows that galactose and α-methylglucoside absorption increases with substrate concentration following kinetics that may be explained by a non saturable linear passive component and another one of saturable mediated transport, phlorizin sensitive. The passive component has been measured by blocking the second one in the presence of 0.5 mM phlorizin, the transport being estimated as the difference between absorption without and with phlorizin.

The passive component for galactose and α-methylglucoside is coincident (parallelism test, 28) with the absorption of the non transportable sugar 2-deoxyglucose, so that the K_D mass transfer coefficient for the three substrates was not significantly different (0.064, 0.063 and 0.067 μmol · cm^{-1} · 4 min^{-1} · mM^{-1}, respectively).

The phlorizin sensitive transport component complies well with the Michaelis-Menten kinetics for just one transport system. The Lineweaver-Burk plot has allowed the obtention of the apparent kinetic constants, which for galactose were V'max = 1.09 μmol · cm^{-1} · 4 min^{-1} and K'm = 21.7 mM, and for α-methylglucoside V'max = 0.95 μmol · cm^{-1} · 4 min^{-1} and K'm = 13.5 mM.

Mutual inhibitions between D-galactose and α-methylglucoside. The inhibition produced by 25 mM α-methylglucoside on the active transport of 0.1 and 40 mM galactose was determined *in vivo* as well as that provoked by 25 mM galactose on the active transport of 0.1 and 40 mM α-methylglucoside. Mutual inhibitions between both sugars were also measured with concentrations of 0.1 and 10 mM for the one acting as substrate and eight times higher for the inhibitor. In every case active transport was measured as the difference in substrate absorption in absence or presence of 0.5 mM phlorizin.

Table I shows the results obtained in the experiments, accompanied by the theoret-

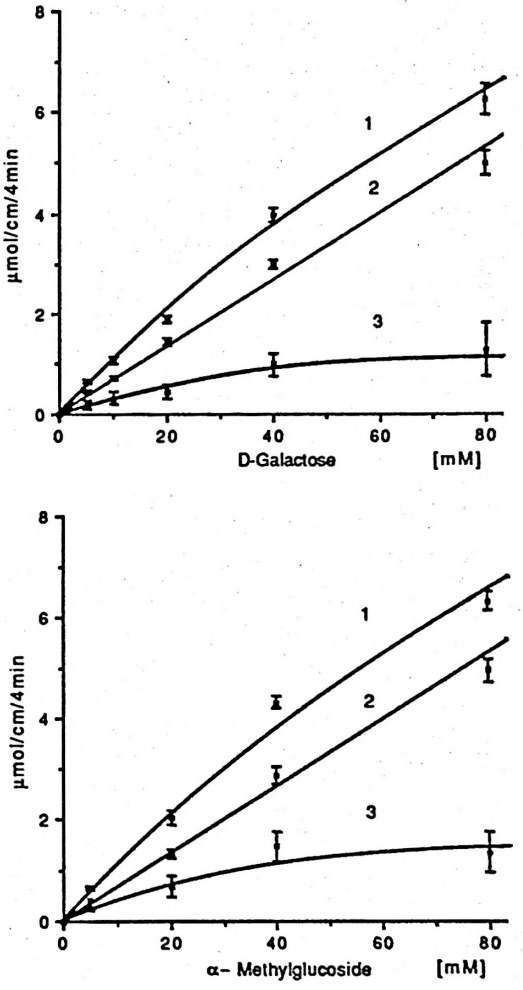

Fig. 1. *Intestinal absorption kinetics of D-galactose and α-methylglucoside in rat jejunum* in vivo. 1) Total absorption; 2) passive diffusion (presence of 0.5 mM phlorizin); 3) active transport. The experiments were carried out with solution (10 ml) recycling at 6.2 ml/min rate and 4 min long absorption periods. The active transport curve was estimated from the difference between total absorption and diffusion. Each point is the mean of 6 to 30 experimental data.

Table I. *Mutual inhibitions on the active transport between galactose and α-methylglucoside in rat jejunum in vivo.*

Transport estimated as the difference between absorption without and with 0.5 mM phlorizin. Experimental values and the estimated ones assuming competitive inhibition for one transport system. Units: μmol/cm/4 min.

		Experimentals		Calculated	
	N	Transport	% Inhibition	Transport	% Inhibition
Gal 0.1 mM	11	0.005 ± 0.0007		0.0050	
Gal 0.1 + MG 25 mM	8	0.001 ± 0.0006	80.0	0.0017	66.0
Gal 40 mM	19	0.98 ± 0.022		0.71	
Gal 40 + MG 25 mM	12	0.63 ± 0.029	35.7	0.43	39.4
MG 0.1 mM	22	0.007 ± 0.0011		0.0070	
MG 0.1 + Gal 25 mM	12	0.002 ± 0.0011	71.4	0.0033	52.9
MG 40 mM	30	1.47 ± 0.30		0.71	
MG 40 + Gal 25 mM	18	0.84 ± 0.31	42.8	0.55	22.5
Gal 0.1 mM	8	0.0049 ± 0.0008		0.0050	
Gal 0.1 + MG 0.8 mM	8	0.0043 ± 0.0008	12.2	0.0047	6.0
Gal 10 mM	9	0.41 ± 0.05		0.34	
Gal 10 + MG 80 mM	7	0.05 ± 0.03	87.8	0.068	80.0
MG 0.1 mM	8	0.0050 ± 0.0004		0.0070	
MG 0.1 + Gal 0.8 mM	6	0.0048 ± 0.0006	4.0	0.0067	4.3
MG 10 mM	10	0.54 ± 0.04		0.40	
MG 10 + Gal 80 mM	10	0.13 ± 0.03	75.9	0.13	67.5

ical values calculated assuming competitive inhibition between two substrates which share the same transport system. The kinetic parameters (V'max and K'm) obtained in the previous section have been used. Although some differences between the experimental inhibitions and the theoretical ones are observed in some cases, the parallelism may be considered acceptable.

Similar experiments of mutual inhibitions were carried out *in vitro* in rat jejunum sacs (figure 2). It was observed that 25 mM α-methylglucoside inhibited 0.1 mM (73.2 %) and 40 mM (62.2 %) galactose in a slightly different proportion, while 25 mM galactose inhibited 0.1 and 40 mM α-methylglucoside transport in 84.1 % and 22.4 % respectively. As in *in vivo* experiments, sugar transport was calculated by subtracting from the total uptake the passive component measured in the presence of 0.5 mM phorizin.

Effect of cytochalasin B. — *In vitro* experiments with rat jejunum sacs showed that 0.1 mM cytochalasin B, present in the mucosal solution with the sugar, inhibits both galactose and α-methylglucoside transport at 0.1 or 40 mM concentrations (figure 3). The inhibitions (in %) are of a similar order both at low and high substrate concentrations.

Effect of low temperature. — To find out if there is in rat jejunum a sugar transport system that ceases to operate at low temperature, transport of 0.1 and 40 mM

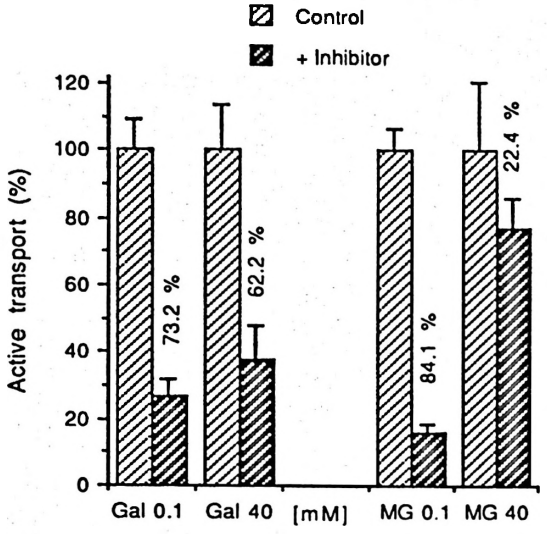

Fig. 2. *Inhibitory effect of 25 mM α-methylglucoside on the active transport of 0.1 and 40 mM galactose and 25 mM galactose on that of 0.1 and 40 mM α-methylglucoside in rat jejunum* in vitro.
Closed empty everted jejunum sacs. 4 min incubation. Units: μmol/g p.f./4 min. Inhibitions in %. n = 12-25. (Gal, Galactose; MG, α-methylglucoside).

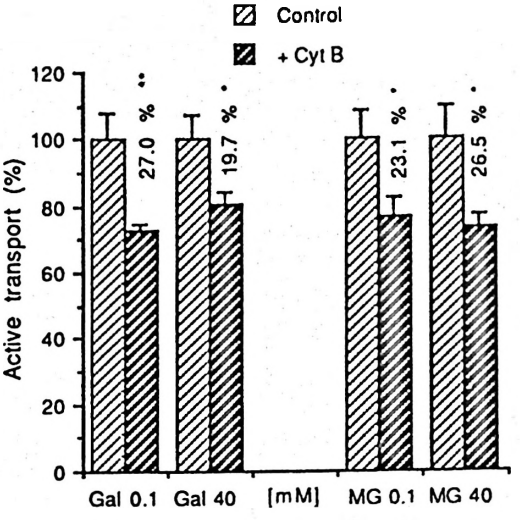

Fig. 3. *Inhibitory effect of cytochalasin B on the active transport of 0.1 and 40 mM galactose and α-methylglucoside in rat jejunum* in vitro.
Experiments, symbols and units as in fig. 2. n = 9-14. * $p < 0.05$; ** $p < 0.01$. (Cyt B, Cytochalasin B).

galactose was measured *in vitro* at the temperatures of 37 and 20 °C. As figure 4 shows there is transport at both temperatures, and it is lower at 20 °C than at 37 °C. The transport inhibition at low temperature is 60.7 % and 77.5 % with galactose concentrations of 0.1 or 40 mM respectively. In the hypothesis of two transport systems of which the one with low affinity and high capacity would cease to operate at the lowest temperature, the transport inhibition of 40 mM galactose should have been considerably higher.

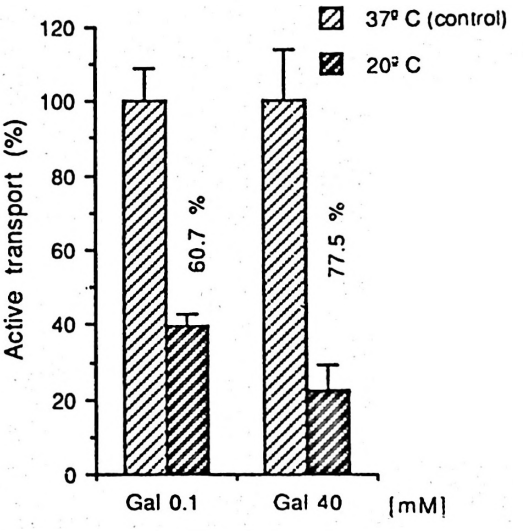

Fig. 4. *Inhibitory effect of low temperature (20 °C) on the active transport of 0.1 and 40 mM galactose in rat jejunum* in vitro.
Experiments, symbols and units as in fig. 2. n = 12-25.

Discussion

The absorption kinetics of galactose and α-methylglucoside by perfused rat jejunum *in vivo* may be explained as the sum

of an active saturable, phlorizin sensitive transport component and another apparently passive linear component which persists in the presence of 0.5 mM phlorizin. In the range of 0.1 and 80 mM concentrations, the active transport of both substrates acceptably follows the Michaelis-Menten kinetics for one transport system allowing the estimation of the corresponding apparent Vmax and Km values. For galactose these parameters are similar to those previously found in parallel experimental conditions (13). The Vmax for the two substrate transports are coincident, which further suggests that they share just one system.

The mass transfer coefficients, K_D, for the passive component of the galactose and α-methylglucoside absorption are practically coincident among themselves and with the non transportable sugar 2-deoxyglucose. This would seem to exclude in the adopted experimental conditions the use of a low affinity and high capacity, phlorizin insensitive, transport system by galactose (2), as in such a case the K_D for that sugar would have to be higher than that for the other substrates and the absorption kinetics in the presence of phlorizin would have to move away from the linearity at high concentrations.

The mutual inhibitions of the transport component between galactose and α-methylglucoside may be interpreted as competitive inhibitions between both substrates for the same transport system. Such results do not demand the existence of two simultaneously operative systems, with very different Km and Vmax, the one with low affinity (SII) not being shared by α-methylglucoside. In fact, if the two systems existed, galactose transport inhibition by 25 mM α-methylglucoside would only affect the high affinity system (SI) so that it would be very high with 0.1 mM galactose and nearly negligible with the 400 fold higher concentration of 40 mM galactose, which does not occur. Equally, under the same assumption of the two operative systems, with α-methylglucoside concentrations eight fold higher than galactose, the experimental data should be much more removed from the calculated ones when the sugar concentration is 10 mM.

Cytochalasin B is an inhibitor of the sugar transport system at the basolateral membrane (16, 17, 25) and at BBMV (1, 2, 26). The *in vitro* experiments with cytochalasin B in rat jejunum sacs revealed quite similar inhibitions (%) of galactose and α-methylglucoside at 0.1 and 40 mM concentrations. It is difficult to explain this result if there existed two systems, of which only the one with low affinity and high capacity would be cytochalasin sensitive, as in that case higher inhibitions should be expected at high galactose concentrations than at low ones, and no α-methylglucoside transport inhibition should be observed since it does not use that system.

The effect of temperature, studied *in vitro* in rat intestine sacs, does not support either the existence of two transport systems of which the one with low affinity would not operate on passing from 37 °C to 25 °C. The inhibition from low temperature (20 °C with respect to 37 °C), should clearly be smaller when galactose concentration is very low (0.1 mM), so that the dominant system is that of high affinity and quite higher with 40 mM galactose, a situation in which the one with low affinity would acquire greater importance. Although our results reveal some inhibition increase at high sugar concentration, the differences are small. On the other hand, inhibitions in sacs are quite higher than those observed in BBMV, which should be attributed to the importance of temperature in cellular processes related to the active transport in rat intestine.

An important objection to the studies of transport kinetics with *in vivo* or *in vitro* techniques with intact tissue is the distorting effect of the unstirred water layers

(UWL), as the concentrations of the substances in the perfusion solution or incubation medium are higher than at the outer surface of the membrane and they also originate errors in the estimation of the passive component, especially at high substrate concentrations. This explains why, with these techniques, apparent Km (K'm) values are obtained higher than the "true" Km ones obtained with BBMV or other preparations with small UWL resistance. Although that is not an obstacle to relate the experimental values of transport and inhibition with those estimated for just one system, it is very important when it comes to discussing the possible contribution of two transport systems of different Km and Vmax. If the UWL cause the real substrate concentration at the membrane to be very much lower than that of the solution, it may happen that even with 40 mM concentrations in the solution the contribution of the low affinity and high capacity system might ve very slight, the possibilities of discriminating between 1 or 2 systems being minimized.

These results, as a whole, do not support the theory that under the adopted experimental conditions, with substrate concentrations close to the physiological ones at the lumen of the rat small intestine (8,13), two transport systems for glucose and analogues of properties similar to those described in BBMV of guinea pig, are operative. This may be attributed to differences in species or to the distorting effects of the UWL.

Resumen

En yeyuno intacto de rata se intenta verificar *in vivo* e *in vitro*, si son operativos los dos sistemas de transporte activo de análogos de glucosa caracterizados en vesículas de membrana del borde en cepillo de yeyuno de cobayo. Tras determinar los componentes pasivo y de transporte de la absorción intestinal de glucosa y del α-metilglucósido, se estudia la cinética de transporte de ambos sustratos, así como las inhibiciones recíprocas entre ellos, a concentraciones bajas y altas del azúcar sustrato y a distintas concentraciones del inhibidor. También se observa *in vitro* el efecto de la citocalasina B y de la temperatura sobre el transporte. La citocalasina B inhibe en % equivalente el transporte de galactosa y de α-metilglucósido tanto 0,1 como 40 mM. A 20 °C, el transporte de galactosa a esas concentraciones resulta inhibido en un 61 y 77 %, respectivamente. El transporte de D-galactosa y de α-metilglucósido resulta 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 un segundo sistema de transporte activo en el borde en cepillo de yeyuno de rata no se pone de manifiesto en preparados intestinales de tejido completo, quizá debido a diferencias de especie animal o a la presencia de capas de agua no agitadas.

Palabras clave: Sistemas de transporte, Galactosa, α-Metilglucósido, Yeyuno de rata, Citocalasina B.

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