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In vivo Increase of Passive Intestinal Absorption by Cotransporters Activation in Rat*

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The effect of the luminal exposure to 25 mmol/L galactose, alanine, tryptophan or sorbitol on the passive absorption of 10 mmol/L galactose, mannitol and 2-deoxyglucose has been studied in *in vivo* perfused rat jejunum. Absorption of mannitol or 2-deoxyglucose is markedly enhanced by the presence of any of the three cotransported substrates, but not by that of the non transportable sorbitol. Furthermore the passive absorption of galactose (measured in the presence of 2 mmol/L phloridzin) increases after alanine active transport, while it remains unmodified after sorbitol exposure. The stimulatory effect on mannitol or 2-deoxyglucose absorption can be observed 5 min after addition of galactose and it is not significantly modified during the 50 min period that followed. The passive absorption increase observed with alanine and especially with galactose seems to be easily reversed by washing with saline solution. With tryptophan, the effect remains after washing but some mucosa desquamation is observed. These results with direct measures confirm the passive absorption stimulation by the activation of Na^+-substrate cotransporters. The passive absorption may take place by the paracellular way through the tight junctions.

Key words: Rat jejunum, Sugar paracellular absorption, Na^+-substrate cotransporter activation.

A portion of the nutrient intestinal absorption after a meal may be passive, preferably carried out by the paracellular pathway. Paracellular permeability seems to be determined by the functional structure of the intercellular tight junctions, (*zonula occludens*, ZO) apically located between the enterocytes, free diffusion occurring in the intercellular space below ZO (6). Intracellular mediators and alterations of the extracellular medium have been observed to alter the tight junction structure in some epithelia, suggesting that cytoskeleton may be involved in transducing such

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messages (4, 6-7). Composition of luminal content in the intestine widely varies during a meal and may lead to glucose concentrations higher than the transport system saturation point (2, 5, 9, 12). Recent findings indicate that the presence of glucose or actively transported amino acids in the intestinal lumen elicits a decrease in transepithelial electrical resistance and an increase in paracellular permeability, due to an alteration of the tight junction structure related to the associate cytoskeletal components (1, 6-8, 11). It has been also reported that the active transport of D-glucose augments the antigen-mediated fluid secretion in rat intestine, possibly by enhancing permeation of the intestinal epithelium to antigen (14).

The present *in vivo* experiments directly confirm that the presence of the Na^+-cotransported substrates galactose, alanine and tryptophan in the perfused lumen of rat small intestine exerts a clear enhancing effect on the passive absorption of galactose, mannitol and 2-deoxyglucose.

Materials and Methods

Male Wistar rats (180-200 g) were anesthetized with pentobarbital sodium, s.c. at 60 mg/Kg. An about 20 cm long segment of proximal jejunum was perfused (peristaltic pump) according to the *in vivo* PONZ *et al.* method (13). Ten mL of the perfusion solution (in mmol/L: 140 NaCl, 5.6 KCl, 3 $CaCl_2$, 2.8 $SO_4Mg \cdot 7\ H_2O$, 2.8 PO_4H_2K, 6.1 TRIS, 4.88 HCl; pH 7.4) containing the labelled (^{14}C) sugar or mannitol were recycled at 5.6 mL/min during 5 min absorption periods. Previous results (13) have shown that under these conditions the absorption rate remains constant throughout the successive periods for at least 120 min. At 5.6 mL/min perfusion rate the unstirred water layer resistance is less important than at lower rates (10). In each animal, the passive absorption of the labelled compounds was measured in three successive experimental conditions: a) control, b) in the presence of an actively cotransported substrate, after 15 min perfusion with saline solutions containing this substrate, c) again in the absence of the transported substrate. Three successive absorption periods for each condition were conducted. At the end of each absorption period, the whole perfusate was collected to determine the amount of non absorbed compound. The jejunum segment was next washed for 2 min with the perfusion saline solution and emptied by air passing. The concentration of the labelled compound was determined by radioactivity counting and the absorption rate was calculated from the difference between the initial and final perfusate contents and expressed in μmol/(cm · 5 min). Each experiment was carried out in the course of a few weeks with a homogeneous group of animals. Differences among the control passive absorption of the several experiments might have been due to the different times of the year when they were carried out and to the various groups of animals used.

All the chemical products were of analytical grade. 1-(^{14}C)-2-deoxy-D-glucose (50.3 μCi/mmol), 1-(^{14}C)-D-mannitol (4.3 μCi/mmol), and 1-(^{14}C)-D-galactose (60 μCi/mmol) were from DuPont. Statistical analysis was made by using paired Student's *t* test and single factor ANOVA with a posteriori Fisher test. A $p<0.05$ was considered statistically significant.

Results

The passive absorption of the non transportable monosaccharide derivatives mannitol (fig. 1) and 2-deoxyglucose (fig. 2) at 10 mmol/L concentration markedly increased (even up to 100 %) when 25 mmol/L galactose or alanine were present in the perfusion solution as described for the experimental condition b). This effect was, however, not observed if the non transportable sorbitol, instead of galactose

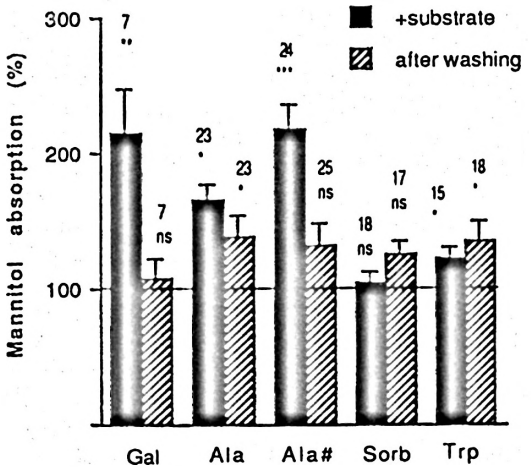

Fig. 1. *Activation of the passive absorption of 10 mmol/L mannitol by the transport of 25 mmol/L galactose, alanine or tryptophan in rat intestine* in vivo.

Results are expressed as percentage (mean ± standard error) of corresponding control values (horizontal line). After measuring the mannitol absorption in control conditions (value 100, which corresponds to an absorption value of 0.48±0.04 μmol/cm · 5 min), it was again determined in the presence of different substrates (galactose (Gal), Ala, sorbitol (Sorb), or Trp) after their perfusion during 15 min (closed column), and lastly, again after 5 min washing in their absence (dashed column). In Ala# experiments, the washing lasted 15 min. On the top of the column, number of experiments. Statistical signification of the differences are referred to the corresponding control values. * $p<0.05$, ** $p<0.01$; *** $p<0.001$; ns, not significant.

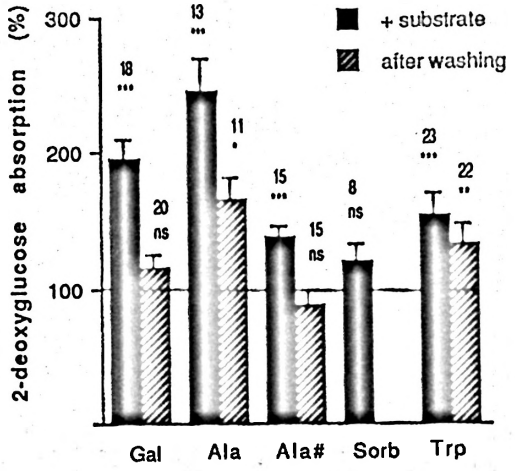

Fig. 2. *Effect of 25 mmol/L galactose, alanine, sorbitol or tryptophan on 10 mmol/L 2-deoxyglucose absorption in rat intestine* in vivo.

Legend as in Fig. 1. Value 100 corresponds to an absorption of 0.450±0.04 μmol/cm · 5 min.

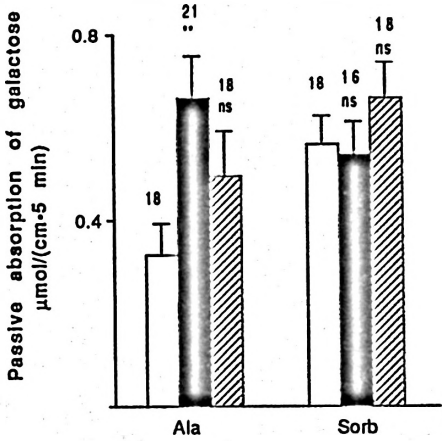

Fig. 3. *Effect of 25 mmol/L alanine or sorbitol on the passive intestinal absorption of 10 mmol/L galactose* in vivo.

Passive absorption of galactose was obtained in the presence of 2 mmol/L phloridzin. It was determined in control conditions (open column), in the presence of alanine or sorbitol, after perfusion with these substrates during 15 min (closed column), and lastly, after 5 min washing, in their absence (dashed column). Results are expressed as mean ± standard error. At the top of the column, number of experiments. Statistical signification of the differences is referred to the corresponding control values. ** $p<0.01$; ns, not significant.

or alanine, was present at the same concentration. Passive absorption of 10 mmol/L galactose, measured in the presence of 2 mmol/L phloridzin to block the Na^+-sugar cotransport, was also enhanced by alanine transport, being unaffected by sorbitol (fig. 3).

The stimulation of the mannitol (fig. 1) and 2-deoxyglucose (fig. 2) passive absorption due to the presence of galactose in the perfusion solution was reversed after 5 min washing with sugar-free saline solution. When alanine was added, the stimulatory effect was also reversed by sufficient wash-

ing with saline solution (figures 1-3). Moreover the perfusion with 25 mmol/L tryptophan seemed to increase mannitol and 2-deoxyglucose absorption (figs. 1 and 2), although a weak desquamation of the mucosa was observed, the effect not being reversed after 5 min washing.

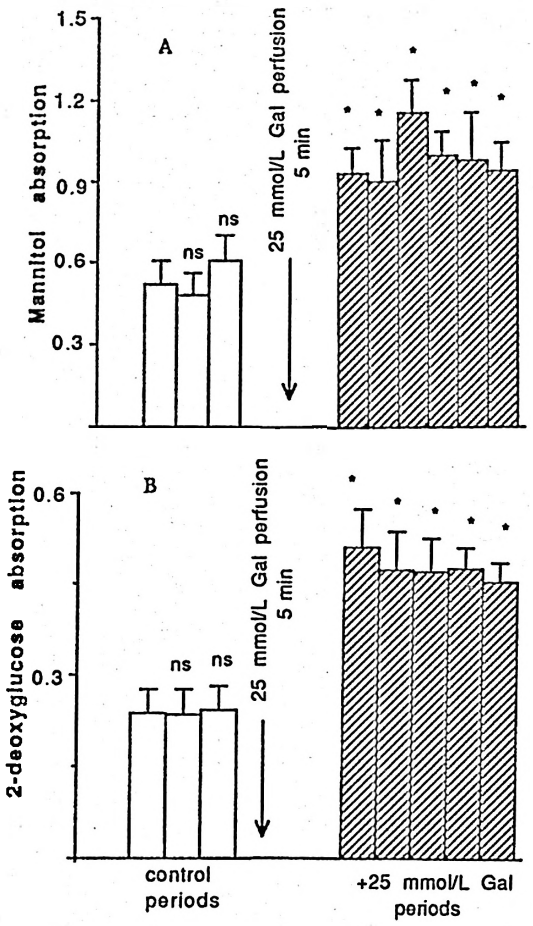

Fig. 4. *Stimulatory effect due to the presence of 25 mmol/L galactose on 10 mmol/L mannitol (A) or 2-deoxyglucose (B) absorption, µmol/(cm · 5 min), in rat intestine* in vivo, *through time.*
After measuring mannitol or 2-deoxyglucose absorption in three successive periods in control conditions, the intestine was perfused for 5 min with 25 mmol/L galactose and then the absorption in the presence of galactose was measured along 6 or 5 successive 5 min periods. Statistical signification is referred to the first control period; * $p < 0.05$. Number of determinations, 9 (A) and 8 (B).

To see if the stimulatory effect of 25 mmol/L galactose present in the perfusion solution changes through time, the absorption of 10 mmol/L mannitol and 10 mM 2-deoxyglucose was measured in other experiments; first in control conditions (three successive 5 min periods), then the lumen was perfused for 5 min with saline containing 25 mmol/L galactose, and lastly the mannitol or 2-deoxyglucose absorption during six or five successive periods was measured again in the presence of galactose. Moreover, the 2 min intermediate washing between these absorption periods was carried out with saline solution containing 25 mmol/L galactose. As Fig. 4 shows, the increasing effect elicited by galactose transport appeared as early as in the first absorption period, i.e. 5 to 10 min after the beginning of the perfusion with the sugar, and did not significantly change through time for at least 50-60 min.

Discussion

The present *in vivo* results reveal that a 15 min perfusion of the rat jejunum lumen with saline solutions containing actively cotransportable substrates at a 25 mmol/L concentration increases sugar passive absorption quite markedly. This increase is not produced when sorbitol is the added substrate, which does not use the transport system. As galactose is poorly metabolized by the intestine epithelial cells, these results indicate that the stimulation of the passive absorption is not due to energetic or other unspecific effects, but rather to the cotransporter activation. This activation, as already pointed out (1), is accompanied by structural tight junction modifications, that may account for the increase in the passive paracellular fluxes. The enhancing effect of D-glucose on the intestinal responsiveness to the antigen has also been related to the activation transport of the

hexose because it diminishes in the presence of phloridzin and is mimicked by β-methylglucoside, but not by L-glucose (14).

The stimulatory effect on passive absorption can be observed after 5-10 min exposure to the transportable substrates and it does not vary significantly if the presence of these substrates is prolonged for up to 50-60 min. Similarly, the decrease in transepithelial electrical resistance and the increase in short circuit current induced in hamster intestine by mucosal addition of glucose, phenylalanine, proline or methionine occur within 5 min and remain for over 30 min (8).

The passive absorption increase disappears after washing with a substrate-free physiological solution when the stimulation was elicited by galactose or alanine, although a longer washing period seems to be required when alanine was used. Also structural studies indicated that the tight junction alteration in response to luminal glucose (6) is easily reversible. With tryptophan, the effect appears with as little as a 5 mM concentration (data not shown) and it seems to remain or even increase after washing. MADARA *et al.* (8), with ultrastructural studies and electrical measures, have also reported an exaggerated lasting response of the intestinal epithelium to the 1-5 mmol/L tryptophan transport, including permeation to macromolecules associated to disorganization of the tight junctions and profound alterations in the cytoskeleton. According to these authors, tryptophan, already at these low but supraphysiological concentrations, exerts a toxic effect on the intestinal epithelium. In our experiments, mucosa desquamation was observed with 25 mmol/L Trp.

The passive sugar absorption increase in response to activation of Na^+-substrate cotransporters has been attributed to an osmotically induced solvent drag through the altered tight junctions (12). In our *in vivo* experimental conditions, the measures of fluid flow towards the serosal side were not accurate enough to explain the whole increase in substrate passive absorption in terms of a proportional increase in fluid volume. Experiments *in vivo* (3) and *in vitro* (not published) in conditions of luminal hyperosmolarity (plus 200 mmol/L mannitol) seem to reveal a passive flux of substrate in spite of a net fluid transfer towards the lumen.

Summing up, the present results with direct measure of the *in vivo* sugar passive absorption confirm the dynamism of the tight junctions and the possibilities of paracellular permeability regulation in response to the luminal content composition. Although there exist wide discrepancies among the authors (2, 5, 9, 12), glucose luminal concentration after a meal may be 25 mmol/L or greater, which practically saturates the usual transport systems. The paracellular passive absorption would, therefore, start becoming important, conferring physiological signification to its increase by the cotransporter activation.

Resumen

Se estudia en yeyuno de rata perfundido *in vivo*, el efecto de la presencia luminal de galactosa, alanina, triptófano o sorbitol (25 mmol/L) sobre la absorción pasiva de la galactosa, manitol y 2-deoxiglucosa (10 mmol/L). La absorción de manitol y de 2-deoxiglucosa aumenta apreciablemente por la presencia de cualquiera de los tres sustratos que utilizan un sistema de transporte asociado a Na^+, pero no en presencia de sorbitol que no utiliza sistema de cotransporte. También la absorción pasiva de galactosa, medida en presencia de florricina 2 mmol/L, aumenta tras la exposición a alanina, sustrato que se cotransporta activamente, pero no se modifica por el sorbitol. El efecto estimulador sobre la absorción de 2-deoxiglucosa y de manitol se observa a partir de 5 minutos de exposición a la galactosa, y no cambia significativamente si se prolonga la exposición hasta 50 minutos. El incremento en la absorción pasiva

observado con alanina y más aún con galactosa revierte fácilmente por lavado del intestino con solución salina. Con triptófano el efecto permanece después del lavado, pero se observa además descamación de la mucosa. Estos resultados confirman la estimulación de la absorción pasiva por la activación de los cotransportadores Na^+-sustrato. La absorción pasiva puede tener lugar por la vía paracelular a través de las uniones estrechas.

Palabras clave: Yeyuno de rata, Absorción paracelular de azúcares, Activación del cotransportador Na^+-sustrato.

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