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Effect of Luminal Na^+ on the Kinetics of Intestinal Absorption of Sugars *in vivo* *

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The effect of sodium concentration on the absorption kinetics of glucose, galactose and 3-*o*-methyl-glucose in rat and hamster jejunum *in vivo* has been studied. In consecutive 1 min periods the total absorption and absorption in presence of 0.5 mM phlorizin were measured. The difference between them was taken as the active transport rate. The perfusion rate value was 5.6 ml · min^{-1} and sugar concentrations in the perfusion solution ranged from 1 to 10 mM. The results for the different sodium concentrations show a nearly common Vmax for the same sugar and animal species, while the apparent K_T values increase when the sodium concentration in the lumen decreases, mimicking a pure affinity-type activation system. The absorption of sugar when solutions without Na^+ are perfused, is greater than that entering passively in the presence of phlorizin. An explanation may be that appreciable amounts of endogenous Na^+ find their way to the intestinal lumen in favour of the gradient, making Na^+-sugar cotransport possible.

Key words: Sodium, Intestine, Jejunum, Sugars, Absorption, Transport, Rats, Hamster.

During the last decades the relation between Na^+ and the transport of sugars and other non-electrolites by the intestinal epithelium has been shown to be highly significant (6, 12, 29, 30). In general cotransport mechanisms for sugars and Na^+ have been inferred from observations such as kinetics data (11, 14, 19), bindings to phlorizin (33, 34), and measurement of intracellular activities of sodium (12).

In *in vitro* experiments, the level of accumulation and the rate of penetration into the epithelial cells are a function of the mucosal concentration of Na^+, which seems to influence the transport apparent constant or its maximum rate (7, 10, 15, 29, 30). In *in vivo* conditions the importance of Na^+ has also been brought out in diverse species (3, 8, 21, 22, 24). In rat and hamster, in 1 minute long absorption periods (22) the absorption of glucose, galactose and 3-*o*-meth-

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ylglucose is clearly inhibited in the absence of Na^+ (70-80 %), and returns to its normal values on being perfused again with sodium. The degree of inhibition varies with the sugar and depends on the substituent employed as well. The absorption of arabinose is not affected by Na^+ (22). Other results *in vivo* had granted lesser importance to luminal Na^+ (4, 28).

As it is known, sugar intestinal absorption *in vivo* requires the diffusion of substrate molecules across the unstirred water layers (UWL) from lumen to the epithelial cell and their transference across the epithelium by a passive component and by mediated transport (9, 23, 36). Previous works (23, 26) have shown the influence of the factors affecting the thickness of the UWL, so that at present the study of the effect of Na^+ on transport kinetics is of high interest, as the presence of the UWL (32) will influence in a decisive way the Na^+ concentration at the level of the enterocyte membrane when the cation has been totally or partially eliminated from the medium.

Materials and Methods

Wistar rats (100-250 g) and golden hamsters (60-140 g) of both sexes were used; they fasted for 24 h before each experiment. The animals were anesthetized with urethane (125 mg/100 g animal weight). A jejunum segment, approximately 20 cm long, was cannulated *in situ*, through which the sugar solution was perfused at 5.6 ml/min rate (25).

Single-pass perfusion was used throughout. Absorption periods were 1 min long. From 5 to 12 successive periods were carried out in the same intestinal segment. Volume changes in the solution on passing through the intestinal segment turned out to be negligible.

Sugar concentrations in Krebs-Ringer-Tris (pH 7.4) solution (17, 35), were between 1 and 10 mM. When necessary, NaCl was replaced by LiCl preserving the osmolarity.

The sugar concentrations in the perfusate at the influx and outflux of the intestine were determined by the Nelson-Somogyi method (19, 31). Absorption values were measured as the disappeared sugar, and are expressed in nmoles $\cdot$ $\cdot cm^{-1} \cdot min^{-1}$ (25). The sugar mediated transport component was calculated by subtracting the absorption with 0.5 mM phlorizin from that without the glycoside. In some cases the Na^+ concentration in the perfusion solution was determined before and after its passage through the jejunum, by flame photometry, using an atomic absorption spectrophotometre (Instrumental Laboratory 451).

Results

Rat. Successive absorptions of D-glucose, D-galactose and 3-*o*-methyl-D-glucose were used at 1, 2, 5 and 10 mM concentrations with Na^+ concentrations of 128 (control), 64, 32 or 0 mEq/l. The intervening washings were carried out with Krebs-Ringer-Tris at the same Na^+ concentration as the one to be used in the next absorption period. The washing was thorough after 0.5 mM phlorizin had been present. Only the calculated values for the transport component of sugar absorption are reported.

Figure 1 shows the mediated transport kinetics as a function of the substrate concentration, in Lineweaver-Burk (16) representation, for the different Na^+ concentrations. It may be observed that with each of the three sugars the straight lines intercept the ordinate axis approximately at the same point, suggesting an identical Vmax value, which would be independent of the Na^+ concentration. On the other hand, the slope, and con-

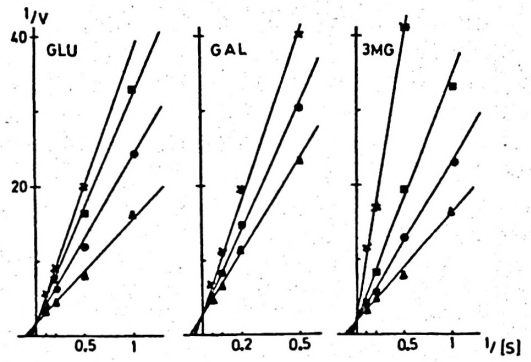

Fig. 1. *Double reciprocal Lineweaver-Burk plot for the transport kinetics of glucose (GLU), galactose (GAL) and 3-o-methyl-glucose (3 MG) in rat jejunum* in vivo *at different* Na^+ *concentrations.*
$[Na^+]$ = ▲ 128, ● 64, ■ 32 and ∗ 0 mEq/l. Abscissa, 1/[s] mM^{-1}. Ordinates, 1/nmole · cm^{-1} · min^{-1}.

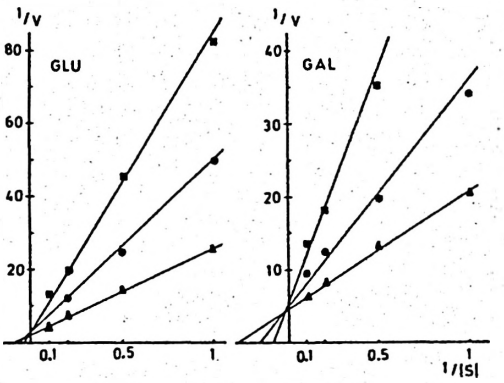

Fig. 2. *Double reciprocal Lineweaver-Burk plot for the transport kinetics of glucose (GLU) and galactose (GAL) in hamster jejunum* in vivo *at different* Na^+ *concentrations.*
$[Na^+]$ = ▲ 128, ● 64 and ■ 32 mEq/l. Abscissa, 1/[s] mM^{-1}. Ordinates, 1/nmole · cm^{-1} min^{-1}.

sequently the K_T apparent values, increases markedly as the Na^+ concentration diminishes. The minimum value for K_T corresponds to D-glucose with 128 mM Na^+. The values for the apparent constants of transport appear in table I.

Hamster. The influence of four distinct Na^+ concentrations (between 0 and 128 mM) on the absorption of 1, 2, 5 and 10 mM D-glucose and D-galactose were studied in the same way as above. Figure 2 shows the double reciprocal

Table I. *Apparent kinetic constants for sugar absorption by rat and hamster jejunum* in vivo *at different* Na^+ *concentrations.*
$[Na^+]$, (mEq/l) · K_T (mM); V_{max} (nmol · cm^{-1} · min^{-1}) for the component of mediated transport.

Sugar	Rat			Hamster	
	Na^+	K_T	V_{max}	K_T	V_{max}
D-glucose	128	9.4	655	8.7	379
	64	17.5	760	11.3	251
	32	22.3	702	15.5	200
	0	23.4	625	18.0	232
D-galactose	128	15.2	364	3.3	209
	64	19.5	352	4.1	151
	32	—	—	7.3	133
	0	23.0	307	10.4	270
3-o-methyl-D-glucose	128	10.2	714		
	64	13.5	629		
	32	16.7	523		
	0	24.1	319		

plot for rate of transport and concentration (16). In table I the K_T and Vmax values for the sugars studied are also included. Just like in rat, the K_T's take higher values when Na^+ decreases in the perfusion solution, whereas the Vmax seems constant.

Discussion

The difference between total absorption and absorption in the presence of 0.5 mM phlorizin may validly be considered as the value for sugar transport (9, 13, 16). The results show that the rate of sugar intestinal transport *in vivo*, both in rat and hamster, is markedly inhibited by the absence of Na^+ from the perfusion solution. The lowest values of the sugar transport constants, are found with Na^+ (128 mM) control concentrations in the perfusion medium. The results obtained from partial Na^+ substitution show that with all sugars the apparent K_T values increase as the sodium concentration decreases, while the Vmax remains constant. This usually happens in *in vitro* preparations with intact epithelium, as if the sugar and Na^+ cotransport system corresponded to an affinity pure type activation kinetics (Type K) (2).

Previous studies with the very same technique (22) revealed that the inhibitions in the absorption of a given sugar due to Na^+ deficiency, depend to a certain extent on the nature of the substituents used (Li^+, K^+, Tris, manitol). In the present work sodium has always been substituted by lithium, whose cation does not seem to bear a direct influence on the enterocytes. According to current ideas, sugar crosses the membrane by means of a sugar, Na^+ cotransport system, which includes a specific binding site for sugar and another one for Na^+ (6, 7, 29). In the absence or with low concentrations of Na^+, sugar affinity for the carrier would diminish. The possibility that the absence of Na^+ might impair the translocation of the complex across the brush border membrane, should not be disregarded either.

The residual absorption observed when sodium free solutions are perfused, is greater than the one produced when the transport has been eliminated by phlorizin. Some remarks suggest that Li^+ can partially substitute Na^+ in the cotransport (18). Another explanation would be that the transport system could translocate the substrate in an appreciable proportion without the Na^+ cotransport. However, in vesicles of brush-border membranes, there is no entry of substrate in the absence of Na^+ (18). As it has been suggested (2), this difference may likewise be attributed to the fact that when the epithelium is intact there is always «a Na^+ apical reservoir» which allows a co-transport to be produced and which would further be responsible for the cotransport kinetics to appear as a K-type, whereas it is really a mixed affinity capacity type (K-Vmax type). The sodium retained next to the brush-border may be of luminal origin from the difficulty of being eliminated by perfusion with a Na^+ free solution, or endogenous, by diffusion towards lumen in favour of gradient, without being totally eliminated from the UWL by the washings between successive absorptions. In fact, with the perfusion technique used in the present work, it has been possible to establish that when the entry solution with 2 mM galactose does not contain sodium, the outflux solution from the jejunum segment possesses a Na concentration of the order of 3 mM. This suggests that in contact with the enterocyte the sodium concentration must be markedly greater. In this way the absorbing cells would have some outside Na^+ available to maintain the sugar, Na cotransport at a certain level.

These results allow us to acceptably reveal *in vivo* a sugar transport kinetics in function of the concentration of Na^+ similar to the one observed in preparations with intact epithelium *in vitro*, while they show the complexity of Na^+ movements across the phases lumen/UWL/cellular membrane, stressing once more the meaning of the unstirred water layers in intestinal absorption kinetic studies in *in vivo* conditions (23, 26, 36).

Resumen

Se estudia en yeyuno de rata y de hamster *in vivo*, el efecto de la concentración de Na^+ sobre la cinética de la absorción de D-glucosa, D-galactosa y 3-*o*-metilglucosa. Se trabaja en períodos sucesivos de absorción de 1 min, midiendo la velocidad total de absorción y la velocidad en presencia de florricina 0,5 mM. Por diferencia se calcula la velocidad de transporte activo. La velocidad de flujo es de 5,6 ml/min y las concentraciones de azúcar entre 1 y 10 mM. Los resultados para las distintas concentraciones de Na^+ muestran un valor aproximadamente común de Vmax para el mismo azúcar y especie animal, mientras que los valores aparentes de K_T aumentan a medida que decrece la concentración de Na^+ en la luz, al modo de un sistema de activación tipo puro de afinidad. La absorción cuando se perfunden soluciones sin Na es mayor que la que entra pasivamente en presencia de florricina. La explicación puede ser que el sodio sale hacia la luz a favor de gradiente y su presencia en las UWL permite cierto nivel de cotransporte de azúcar y Na^+.

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