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Competitive Kinetics in the Inhibition of Sugar Intestinal Transport by Phlorizin, *in vivo* *

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Kinetics of sugar transport inhibition by phlorizin in rat intestine has been studied *in vivo*. Jejunum lumen was perfused during a series of succesive 1 minute periods of absorption. Total sugar absorption was assumed to be the sum of a mediated transport component plus a passive one persisting in the presence of 5×10^{-4} M phlorizin. The values of transport rates, corrected in this way, showed Michaelis-Menten kinetics and proved that inhibition of D-glucose, D-galactose and 6-deoxy-D-glucose transport by low concentrations of phlorizin (5×10^{-6} M) displays a typical competitive kinetics (apparent $K_i = 4\text{-}10 \times 10^{-6}$ M) as had been long before reported from *in vitro* experiments.

Inhibition of sugar intestinal absorption by phlorizin was observed very early (24). From 1946-1955 phlorizin, when in luminal solution, was reported to inhibit strongly sugar absorption *in vivo* across the small intestine even at a 2×10^{-6} M concentration, the inhibition being entirely reversed by the simple expedient of washing out and refilling the intestinal loop with fresh glucose solution (30-32). So easy a reversibility suggested that phlorizin acted at low concentrations just at the luminal surface of the membrane, on loci related to the sugar active transport process (30, 31). The *in vivo* inhibition by 10^{-6} M phlorizin was subsequently confirmed (18, 26), as well as its easy reversibility, *in vitro* (28), Somewhat later phlorizin was demonstrated to act in hamster intestine *in vitro*, as a competitive inhibitor for the sugar transport (4), with very low K_i values. These results have been largely corroborated and extended to other sugars and animals in different *in vitro* preparations including brush border vesicles (1-3, 5-8, 14, 16, 20, 34, 36).

Due to methodological difficulties for kinetic studies, the competitive character of phlorizin inhibition, has not been veri-

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fied as yet under the more physiological conditions of *in vivo* experiments, however, the present research furnishes enough evidence for competitive inhibition kinetics, if sugar transport rates by rat jejunum *in vivo* are taken as the differences between sugar total absorption rates and those in the presence of 5×10^{-4} M phlorizin (11, 12, 15, 17, 23, 27).

Materials and Methods

The experiments were carried out on male and female Wistar albino rats (125-250 g) fasted for 24 hours and anaesthetized with urethane (1.2 g per kg). Absorption rate was measured as described by PONZ *et al.* (29) on *in situ* cannulated jejunum loops (about 20 cm length) which were single pass perfused at a rate of 5.6 ml per minute, with Krebs-Ringer-Tris solution (Tris in place of phosphate, pH 7.4) (37) at 37° C, containing either D-glucose (Merck), D-galactose (Merck) or 6-deoxy-D-glucose (Sigma).

In each animal the sugar absorbed along 18 consecutive 1 minute periods was determined in the following serial order: 3 control periods at three different sugar concentrations; 6 periods at the same concentrations of sugar but in the presence of 5×10^{-6} M phlorizin (duplicate) and other 6 with 5×10^{-4} M phlorizin (duplicate); and finally 3 further control periods at the first three sugar concentrations. Between two consecutive absorption periods the circuit was washed with saline solution and was then cleared of its fluid. Duplicates both for control as for phlorizin, were enough coincident.

Absorption rate was expressed in n-moles of sugar disappeared from the solution per minute per centimeter of jejunum length. Sugar was chemically estimated by the method of NELSON (25) as modified by SOMOGYI (33).

Absorption in the presence of 5×10^{-4} M phlorizin has been taken as equivalent to the passive component. Mediated transport rate has bee calculated as the difference between absorption either in the absence of phlorizin or in the presence of 5×10^{-6} M phlorizin and the passive component value at the same concentration of sugar.

Results

Mean values of absorption rates for D-glucose, D-galactose and 6-deoxy-D-glucose in the absence, V, and in the presence of 5×10^{-6} H phlorizin, V_{ph}, 5×10^{-4} M (V_D) phlorizin concentrations are listed in table I. Sugar concentrations in the perfusion solution were 2, 5, 10 and 20 mM for glucose and galactose, and 1, 2 and 5 mM for 6-deoxy-glucose. The absorption rate for these three sugars in the presence of 5×10^{-4} M phlorizin, V_D, became nearly a linear function of substrate concentration, as should be expected for a passive process. Control absorption, V, and absorption in the presence of 5×10^{-6} H phlorizin, V_{ph}, did not show such linearity, as they included a non-passive saturable component.

From experimental data in table I, transport rates, corrected for the diffusion component, have been calculated as $V-V_D$ for sugar in the absence of phlorizin and as $V_{ph}-V_D$ for sugar in the presence of 5×10^{-6} M phlorizin.

Figure 1 shows the Lineweaver-Burk double reciprocal plots for the transport rate values corresponding to different concentrations of the three sugars. Straight lines, adjusted to experimental points according to the least squares method, fit well the saturation kinetics in the absence of the inhibitor, as well as the competitive inhibition kinetics on addition of 5×10^{-6} M phlorizin. With glucose, galactose and 6-deoxy-glucose, both control and phlorizin lines intercept ordinate axis at the same $1/V_{max}$ point.

The same figure presents the apparent kinetic parameters V_{max}, K_m and K_i values for the different sugars as deduced from the linear representation. K_i has been obtained from $K_a = K_m (1 - [I]/K_i)$, where K_a is the reciprocal of the phlorizin line intercept on the abscissa axis.

Results evidence that inhibition of intestinal sugar transport by phlorizin *in vivo*, exhibits a competitive kinetics.

Table I. *Intestinal absorption of sugars by rat jejunum* in vivo.
Single pass perfusion at 5.6 ml · min^{-1}, absorption periods of 1 min. Absorption rates (nmoles · cm^{-1} · min^{-1}) control (V), and in the presence of 5×10^{-6} M (V_{ph}) or 5×10^{-4} M (V_D) phlorizin. Mean values ± s.e.; number of data in parentheses.

	2 mM	5 mM	10 mM	20 mM
D-glucose				
V	98 ± 5 (8)	209 ± 18 (11)	429 ± 29 (10)	706 ± 26 (7)
V_{ph}	76 ± 9 (5)	173 ± 11 (13)	378 ± 41 (13)	617 ± 77 (6)
V_D	47 ± 5 (6)	107 ± 12 (9)	255 ± 46 (8)	449 ± 36 (5)
D-galactose				
V	114.6 ± 1.8 (10)	248.3 ± 6.6 (18)	478.4 ± 12.3 (19)	803 ± 14.5 (10)
V_{ph}	88 ± 2.3 (11)	200 ± 7.3 (25)	403 ± 18.9 (18)	704 ± 16 (10)
V_D	55 ± 2 (11)	132.5 ± 6 (15)	294.4 ± 10 (21)	530 ± 15 (9)
6-Deoxy-D-glucose	1 mM	2 mM	5 mM	
V	56.2 (3)	81.8 (3)	180 (2)	
V_{ph}	51.8 (3)	72.2 (2)	166.9 (2)	
V_D	40 (2)	50 (5)	113.7 (2)	

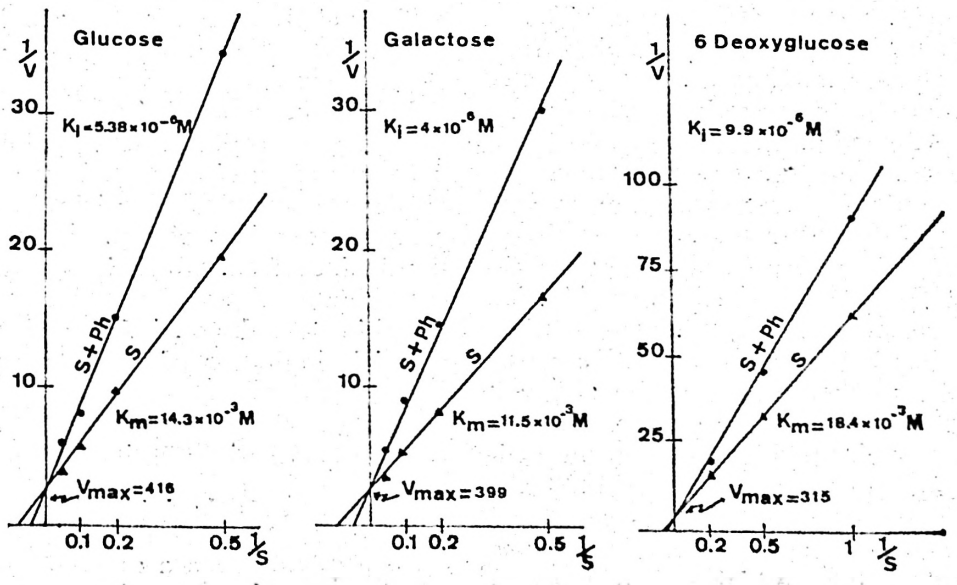

Fig. 1. *Double reciprocal plots of sugar transport by rat small intestine,* in vivo. Data corrected as explained in the text. S, sugar without phlorizin. S+Ph, sugar plus 5×10^{-6} M phlorizin.

Discussion

Intestinal absorption *in vivo* or glucose analogues in the presence of 5×10^{-4} M phlorizin must be attributed either to a passive sugar transfer across the epithelium by a transcellular or intercellular pathway, or to a mediated transport by a phlorizin-unsensitive system with scant affinity for the substrate. At any rate, this component appears to be, in the concentration range used in these and other experiments, in linear relationship to substrate concentration, and accounts for a far from negligible portion of the sugar absorbed under normal conditions, especially as the luminal sugar concentration increases (11, 12, 15, 17, 23, 27).

In order to estimate, therefore, sugar transport rates across the intestine, where a non-passive and a passive component (35, 38) are assumed to exist, the experimental data for total sugar disappeared from the luminal solution have to be corrected for the passive component by substracting the sugar absorbed at the same substrate concentration in the presence of 5×10^{-4} M phlorizin. The *in vivo* sugar transport values obtained from this procedure, unlike the non corrected ones, agree with saturation kinetics under adequate conditions.

The resultant transport rates across rat jejunum for glucose, galactose and 6-deoxy-glucose, after the afore-stated correction displayed a striking approximation to the Michaelis-Menten kinetics, which allowed the correspondent Lineweaver-Burk linear representation to be plotted and the values for the apparent kinetic parameters, to be estimated. The values thus obtained for the apparent semisaturation constants, K_m, were 14.3 mM for glucose, 11.5 mM for galactose and 18.4 mM 6-deoxy-glucose, nearly one order of magnitude higher than those reported from *in vitro* experiments (5, 6, 9, 13, 19), a well known difference accounted by the *in vivo/in vitro* dissimilar experimental conditions, especially related to the greater thickness of the unstirred water layers *in vivo* (10, 21, 27, 39). On the other hand, these apparent K_m values are quite similar to previous ones obtained in the same laboratory (17, 27) at the same perfusion rate.

The calculated V_{max} values were 416, 399 and 315 nmoles$\cdot$cm$^{-1}\cdot$min^{-1} for glucose, galactose and 6-deoxy-glucose respectively. V_{max} values seem to be unaffected by changes in the perfusion rate (27).

In the presence of 5×10^{-6} M phlorizin, plots of 1/V versus 1/S showed the typical competitive inhibition kinetics of sugar transport by phlorizin. The correlation coefficients for the outlines reported in figure 1, in the case of the three tested sugars are sufficiently high, while the point of the intercept on the ordinate axis clearly coincides. Estimations of apparent K_i for phlorizin yielded values of 5.4×10^{-6} M, 4×10^{-6} M and 9.9×10^{-6} M with glucose, galactose and 6-deoxy-glucose respectively, which are close to those calculated from *in vitro* experiments (4, 6,13), and supports the reported high affinity of phlorizin for the glucose transport system.

It may be concluded that when sugar intestinal absorption data supplied by conventional *in vivo* methods are corrected for the passive phlorizin insensitive component, values of transport rates can be obtained from which it is possible to corroborate *in vivo* the typical competitive kinetics observed *in vitro* for the phlorizin inhibition of sugar transport.

Resumen

Se estudia *in vivo* la cinética de la inhibición por florricina del transporte de azúcares por intestino delgado de rata con perfusión luminal, durante períodos sucesivos de 1 minuto. La absorción total se considera la suma de un transporte mediado, más un componente pasivo que persiste en presencia de florricina

5×10^{-4} M. La velocidad de transporte, corregida de este modo, muestra cinética de Michaelis-Menten, y revela que la inhibición del transporte de glucosa, galactosa y 6-deoxi-glucosa por bajas concentraciones de florricina (5×10^{-6} M) exhibe una típica cinética competitiva (K_I aparente $= 4 - 10 \times 10^{-6}$ M) como había sido observada en experimentación *in vitro*.

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