Biochimica et Biophysica Acta, 367 (1974) 371–374

BBA 76778

EFFECT OF PHLORIZIN ON GALACTOSE INFLUX IN RABBIT INTESTINE

J. A. ESTEP and A. M. GOLDNER

Department of Human Physiology, School of Medicine, University of California, Davis, Calif. 95616 (U.S.A.)

(Received June 11th, 1974)

SUMMARY

Direct measurements of the effects of phlorizin and phloretin on the influx of D-galactose across the mucosal brush border of the rabbit jejunum indicates that phlorizin is the competitive inhibitor of the sugar entry process.

The β-glucoside, phlorizin, has been used extensively as an inhibitor of sugar transport across cell membranes in the proximal renal tubule, isolated kidney brush border preparations, erythrocytes and in various intestinal preparations. Diedrich [1] and Malathi and Crane [2] have described an intestinal enzyme not found in the kidney [3] which catalyzes the hydrolysis of phlorizin (K_m, 10^{-4} M) to phloretin and glucose. While it has been shown that phlorizin inhibits sugar transport in the kidney [3], the presence of phlorizin hydrolase (a β-glucosidase) in the intestine has led to considerable controversy as to whether phlorizin or its aglycone, phloretin, is the major sugar transport inhibitor in the intestine. In particular, Columbo and Semenza [4] have shown that phlorizin inhibition of 6-deoxy-D-glucose uptake by rat intestine was a time-dependent process; the extent of inhibition increased with incubation time, suggesting that a phlorizin derivative such as glucose or phloretin rather than phlorizin was the actual transport inhibitor. Phloretin is a more lipid-soluble compound than phlorizin and may penetrate the cell membrane more readily. In the erythrocyte, phloretin is the effective inhibitor and the sugar transport system is relatively insensitive to phlorizin at the same concentration [5]. Consequently, it has been proposed that the actual inhibitor of sugar transport in the intestine is phloretin formed by the hydrolysis of phlorizin. Since many previous studies have failed to distinguish between sugar fluxes across the mucosal and serosal borders of the epithelial cells and have not distinguished between net uptake and unidirectional uptake of sugar, the question of inhibition by externally added phlorizin in the intestine requires further investigation.

In the present experiments, the effect of phlorizin, phloretin, and glucose on the unidirectional D-galactose influx from the mucosal solution into the epithelial cell layer was examined. The results indicate that phlorizin, and not phloretin, is the effective inhibitor of sugar entry into the mucosal epithelial cells of rabbit jejunum.

Unidirectional influxes of D-galactose from the mucosal solution into the cell were determined with the method described by Schultz et al. [6]. The mucosal surface

of rabbit jejunum from male New Zealand white rabbits (sacrificed by intravenous injections of pentabarbitol) was exposed for 60 s to a solution containing D-[^{14}C]galactose in a special chamber and influx was estimated by determining the amount of D-[^{14}C]galactose taken up by the tissue. The inhibitors were only present in the test solutions. [^{3}H] Inulin was used to measure contamination of the surface by adherent test solution. Tissues were preincubated for 30 min in buffered medium containing 140 mM Na^+ or in medium in which all Na^+ was replaced by choline. Preincubation solutions contained no sugar. Influx was measured from solutions containing 140 mM Na^+ or from solutions free of Na^+. All solutions were bubbled with O_2–CO_2 (95 : 5, v/v) to maintain a pH of 7.2 and contained the following: 140 mM NaCl (unless otherwise noted), 10.0 mM $KHCO_3$, 1.2 mM $CaCl_2$, 1.2 mM $MgCl_2$, 1.2 mM K_2HPO_4, 0.2 mM KH_2PO_4. Osmolarity of mucosal solution was maintained constant by appropriate addition of mannitol. D-[^{14}C]Galactose uptake is a linear function of time for at least 80 s and the line extrapolates through the origin. These results indicate that 1 min uptake provides a valid measurement of the unidirectional influx (i.e. initial rate of uptake) of D-galactose. All data presented here were obtained from experiments in which the duration of exposure was less than 1 min and in which each influx measurement was determined in duplicate with concurrent controls, on tissue from the same animal.

Inhibition of influx of D-galactose by phlorizin, phloretin, and D-glucose in the presence and absence of Na^+ are summarized in Table I. In normal buffer, there is a substantial inhibition of influx in the presence of phlorizin and a very slight inhibition in the presence of phloretin. D-glucose shows no inhibitory effect at this concentration. The concentrations of glucose and phloretin are the concentrations expected if all of the phlorizin were hydrolysed by phlorizin hydrolase. In the absence of Na^+ all influx values are not statistically different, regardless of inhibitor.

Experiments on the effect of increasing concentrations of phlorizin on unidirectional influx of D-galactose (8 mM) are summarized in Fig. 1. The data clearly describes a straight line in a plot of the ratio of uninhibited influx to inhibited influx versus the inhibitor concentration and indicates that phlorizin inhibition of D-galactose conforms to competitive and fully reversible inhibition with a calculated K_i of 0.02 mM, which is in qualitative agreement with previously reported results [7].

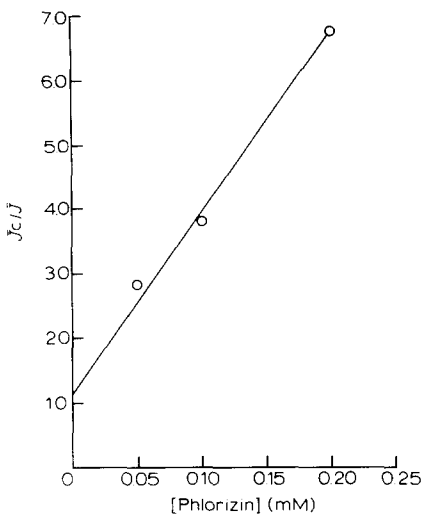
TABLE I

INFLUX OF 8 mM D-GALACTOSE ($\mu M \cdot cm^{-2} \cdot h^{-1}$)

The concentration of D-galactose in all the above experiments was 8 mM. The control value represents the uninhibited influx of 8 mM D-galactose. The other values represent influx in the presence of the indicated inhibitor concentrations.

Buffer	Control	Phlorizin, 0.1 mM	Phloretin, 0.1 mM	Glucose, 0.1 mM
140 mM Na^+ in medium	1.76±0.08 (17)	0.44±0.03* (11)	1.43±0.08* (8)	1.88±0.08 (5)
Na^+ free in medium	0.023±0.01 (6)	0.022±0.01 (8)	0.024±0.01 (10)	

* Significantly different from control at $P < 0.001$.



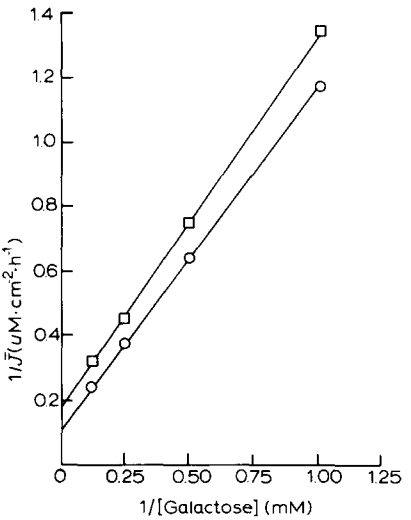


Fig. 1. Kinetics of phlorizin inhibition. Ratio of uninhibited to inhibited D-galactose influx ($\bar{J}_c/\bar{J}$) is plotted against phlorizin concentrations. D-galactose concentration was 8 mM. Calculated K_i for phlorizin is 0.019 M. [Na^+] is 140 mM. K_t for D-galactose is 10.8 mM.

Fig. 2. Lineweaver–Burke plot of uninhibited (○) and inhibited (□) D-galactose influx into rabbit jejunum. Phloretin concentration was 10^{-3} M. [Na^+] is 140 mM. Calculated K_i for phloretin is 0.12 mM. Each point is the average of 3 paired estimations. Paired analysis (Student's t test) shows the regressed lines to be significantly different ($P < 0.05$).

The graph in Fig. 2 shows the effect of 1 mM phloretin on D-galactose influx at 1, 2, 4, 8 mM galactose concentrations. Phloretin caused a small but consistent inhibition of D-galactose influx that is significant ($P<0.05$) by paired analysis (Student's t-test). This suggests an uncompetitive inhibition by phloretin of a small magnitude with a calculated K_i of 0.17 mM. This is in agreement with data presented in Table I indicating a very slight inhibition by phloretin.

The present results provide very strong kinetic evidence that phlorizin is the major inhibitor of D-galactose transport in the jejunum of the rabbit. D-Galactose influx has been shown to be a carrier mediated Na^+-dependent transport system [8]. A comparison of the calculated K_i values for phlorizin and phloretin show that even if the phlorizin in the test solutions were completely broken down into phloretin and glucose by the presence of phlorizin hydrolase, which is present on the mucosal surface of the intestine, the resulting inhibition by the aglycone would be almost 100 times less than the inhibition due to phlorizin. The data in Fig. 1 shows phlorizin to be a fully competitive inhibitor, while the data in Fig. 2 suggest that the relatively small inhibition of D-galactose due to phloretin is of the uncompetitive type. In view of the demonstrated time dependence of phloretin [4, 7] and the results of the present experiments, it is suggested that phlorizin is the major inhibitor of initial D-galactose influx in rabbit jejunum and that phloretin, if it is generated in this tissue preparation, inhibits allosterically or acts as a metabolic poison which does not directly affect the sugar entry process.

ACKNOWLEDGEMENT

This research was supported by U.S. Public Health Service Grant AM 14990

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