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Paracellular Absorption of D-Glucose by Rat Small Intestine *in vivo* *

M. P. Vinardell and J. Bolufer **

Departamento de Fisiología Animal
Facultad de Farmacia
Universidad de Barcelona
Barcelona-28 (Spain)

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D-glucose diffusion in both jejunum and ileum using a perfusion system *in vivo* was determined.

2,4,6-triaminopyrimidine (20 mM) induced an inhibition on D-glucose diffusion of 32 % in the two segments of the small intestine studied.

Glucose net efflux from the jejunum into the lumen was higher than that from the ileum. Phlorizin increased the sugar efflux in both areas.

When an actively transported sugar is perfused *in vivo*, the proportion of the substrate that is absorbed by a diffusion-like process depends on its initial concentration in the perfusate (3, 7, 15), the remnants of substrate being absorbed by active transport.

In this way, it has been proposed that the active pathway starts to play a significant role when substrate concentration in the lumen falls below that of the plasma, thus transporting the substrate that might return to the lumen by leakage (4, 7). However, unequivocal proof about the diffusion pathway, either transcellular or paracellular, in rat intestine is lacking. The aim of this work is to distinguish between the two possible routes.

Materials and Methods

Wistar rats 2-3 months old were used. Animals were fed a standard chow (U.A.R. A.03) and water *ad libitum*. 18 hours before the experiments food but not water was withdrawn. Rats were anesthetized with urethane (1 ml/100 g body weigth, 12.5% solution).

Single-pass or multiple-pass (volume perfusate 10 ml) perfusions (12) of jejunal and ileal loops, with 0.9% NaCl containing D-glucose and ^{14}C-PEG 4000, to determine net water movement, were used. In some experiments phlorizin 5×10^{-4} M and 2,4,6-triaminopyrimidine

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** Departamento de Fisiología Animal. Facultad de Farmacia. Sevilla (Spain).

(TAP) 20 mM were added to the perfusate.

After cannulation, the loops were rinsed and replaced inside the body wall. They were perfused at a flow rate of 3.5 ml/min, with prewarmed (37° C) perfusate. The animals were mantained under controlled temperature.

Glucose was determined by the NELSON (11) and SOMOGYI (16) method and ^{14}C-PEG 4000 in a liquid scintillation counter. The intestinal segment was opened longitudinally and the area was determined at the end of the experiment. The results were expressed as a function of the intestinal area and the experimental time.

Results

The jejunal absorption of D-glucose plotted against its concentration in the perfusate is shown in figure 1. Figure 2 shows the effects of TAP on glucose diffusive component in jejunum and ileum. Multiple-pass perfusions of 10 ml perfusate at a flow rate of 3.5 ml/min during 5 minutes were done. D-glucose concentration was 20 mM and alternatively 20 mM TAP was added. In all perfusions phlorizin 5×10^{-4} M was present in order to avoid the saturable component of absorption.

In other experiments, perfusions of 0.9 % NaCl and with or without 5×10^{-4} M phlorizin were performed during 30 minutes. Results of glucose net efflux from the intestine into the lumen are shown in the Table I.

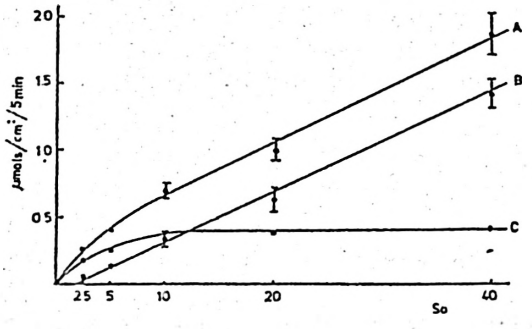

Fig. 1. *Absorption of D-glucose from the perfusate in the absence (curve A) or presence (curve B) of phlorizin 5×10^{-4} M, during single-pass perfusions (flow rate of 3.5 ml/min).*
Ordinate represents the sugar absorption and the abscissa the initial concentration in the perfusate (So). Curve C was calculated by the difference between curves A and B and indicates active transport. The experimental points are the mean ± S.E.M. of eight animals.

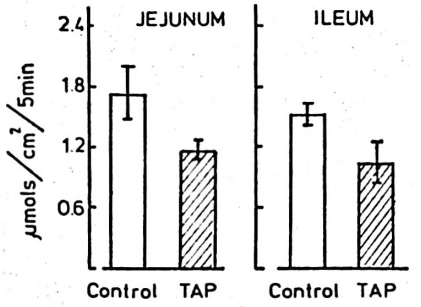

Fig. 2. *D-glucose diffusion by rat jejunum and ileum* in vivo. *Effect of 2,4,6-triaminopyrimidine 20 mM.*
Multiple-pass perfusions at a rate of 3.5 ml/min and succesive absorption periods of 5 minutes were made. D-Glucose 20 mM and phlorizin 5×10^{-4} M were present in all perfusions. Results are the mean ± S.E.M. of six animals.

Table I. *Effect of phlorizin on D-glucose efflux from the rat intestine into the lumen.*
Multiple-pass perfusions at 3.5 ml/min, flow rate of 0.9 % NaCl were used. Results are expressed as nmols/cm²/30 min. The data are mean ± S.E.M. Number of animals in brackets. Statistical significance: a vs b $p < 0.001$ and c vs d $p < 0.001$.

	Jejunum	Ileum
Control	32.9 ± 6.2 (10)[a]	18.6 ± 3.4 (8)[c]
Phlorizin 5×10^{-4} M	104.1 ± 3.6 (6)[b]	65.3 ± 6.3 (6)[d]
Increase efflux %	316	351

Discussion

The jejunal absorption of D-glucose has two components (fig. 1). When the saturable component is blocked by phlorizin 5×10^{-4} M, the sugar absorption rate shows a linear relationship with its concentration in the perfusate. These results agree with previous observations in rat (3, 7, 13, 15), hamster (7, 15), dog (1) and frog (8). Although there is no doubt that the diffusive component of absorption has a significant role, principally in the proximal intestine, the pathway of this process is not clearly established. Two possibilities exist, namely pracellular and transcellular.

FROMTER and DIAMOND (5) classified the small intestine as a leaky epithelium, and other workers showed that the tight-junctions were permeable to lanthanum (9). On the other hand, previous papers showed that TAP blocked monovalent cation transport via the tight-junctions in gallbladder (10), rabbit ileum (6) and rat colon (17), *in vitro*. In our results (fig. 2) TAP inhibits glucose diffusion in jejunum and ileum to the same extent (32%), which indicates that the sugar passing by paracellular pathway was at least 32% of the total, and possibly greater since it is not sure that TAP 20 mM completely blocks the tight-junctions. These results agree with those previously reported *in vitro* in frog (2) and rabbit ileum (14).

Results of glucose net efflux from the blood and intestine into the lumen are shown in the table I. The sugar enters into the lumen at a slow rate. Phlorizin added to the lumen increases the amount of D-glucose in the perfusate, as it inhibits the sugar re-uptake. Similar results in frog intestine (2) have been interpreted as suggesting an efflux route in part paracellular. Phlorizin inhibition of glucose efflux across the brush border must be more than compensated by the inhibition of the re-uptake and for this reason that efflux may be, in part, by paracellular pathway.

The glucose efflux induced by phlorizin seems to be greater in the ileum (351%) than in the jejunum (316%). If it is assumed that the two intestinal segments are leaky to the same extent (fig. 2) this may indicate that re-uptake is more efficient in the ileum, as can be expected taking into account its physiological role in the complete absorption of sugars derived from food in normal animals.

Resumen

Se estudia la difusión de D-glucosa a través del yeyuno y del íleon de rata mediante un sistema de perfusión *in vivo*. La adición de 2,4,6-triaminopirimidina (20 mM) provoca una inhibición del 32% en la difusión de D-glucosa en los dos tramos del intestino delgado estudiados.

El flujo neto D-glucosa desde el yeyuno hacia el lumen es superior al existente desde el íleon. La florricina adicionada en el lumen provoca un aumento de la salida del azúcar en ambos segmentos.

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