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Influence of Temperature of the Perfusion Solution on the Kinetics of Intestinal Absorption of Glucose in Rats

M. J. Rodríguez, M. Ortiz, A. Vázquez, M. Lluch and F. Ponz

Departamento de Investigaciones Fisiológicas del C.S.I.C.
Universidad de Navarra
Pamplona (Spain)

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The kinetics of glucose intestinal absorption by rat jejunum at different solution temperatures, under single pass perfusion, has been studied *in vivo*. The non-passive component (active transport) has been measured as difference between control absorption and that in the presence of 5×10^{-4} M phloridzin. Results at 37° and 39° C did not significantly differ, but at 32° C a clear drop in total absorption, non-passive and passive components was observed. As temperature increases from 32° to 37° C the apparent value of K_m for glucose transport decreases (from 21.2 mM to 13.1 mM), whilst the V_{max} does not change, and the apparent mass-transfer coefficient for the passive component sligtly increases. The results are discussed taking in consideration the effect of temperature on the diffusion across the unstirred water layers and on the active transport processes.

Sugar intestinal absorption *in vivo* requires diffusion of the substrate molecules through unstirred water layers (UWL) from the lumen to the epithelial cell surface, and then their transfer across the epithelium by a passive non-saturable component and a non-passive carrier mediated transport (1, 13 cfr. 6 for other references). Different factors, such as perfusion rate, influence the effective thickness of the unstirred layers and thus the resistance to the diffusion of the substrate across UWL, a process that in physiological conditions seems to be rate-limitant for intestinal absorption (2, 8, 10, 12).

As the temperature of the perfusion solution can affect both the diffusion and the carrier-mediated transport rates, the kinetics of intestinal absorption of glucose by rat jejunum at different temperatures of the perfusion solution has been studied.

Materials and Methods

Male and female Wistar rats of 100-250 g body weight, fasted for 24 h before the experiments were used. Intestinal ab-

sorption was measured according to the PONZ *et al.* technique (7), under urethane anaesthesia (125 mg per 100 g b.w.) in proximal jejunum segments (17-20 cm length) cannulated at both ends. Perfusion was driven by a peristaltic pump (Harvard Apparatus, mod. 1201). The fluid temperature entering the intestine was adjusted to the desired value (39°, 37° or 32° C) by intercalating a coil immersed in a thermoregulated bath in the circuit. Krebs-Ringer-Tris solution (11) at 7.4 pH, with 2, 5, 10 or 20 mM D-glucose, was perfused in single pass through the jejunal segment at a rate of 5.6 ml·min^{-1}. In the same animal the absorption along 12 to 16 consecutive 1 minute periods was measured, the experimental conditions within the series being randomized. The perfusion at different temperatures did not appreciably affect rectal temperature.

D-glucose was determined by NELSON-SOMOGYI method (5, 9). The non-passive transport component (V_T) has been measured (1, 6) as the difference between absorption in the absence (V) and presence of 5×10^{-4} M phloridzin (V_D). Absorption rates are expressed as nanomoles per minute per centimeter of jejunum length (7).

Results

Table I shows the values for total absorption (V), diffusion component (V_D) and transport component (V_T) at the three temperatures of the perfusion solution and at the different concentrations of glucose. At the same temperature V_D increases with acceptable proportionality to the sugar concentration, while V_T follows a saturation kinetics.

Differences between values at 37° and 39° C were not significant. Instead, at 32° C highly significant drops in V_D, V_T and V were observed.

Figure 1 shows the lineal Lineweaver-Burk plot (4) of reciprocal transport rates and sugar concentrations at 32°, 37° and 39° C. The three straight lines drawn according to the least square method intercept ordinate axis at the same point, corresponding to a V_{max} of about 370 nmoles·cm^{-1}·min^{-1}.

Table I. *Glucose absorption by rat jejunum at different temperatures of perfusion solution.* Perfusion rate, 5.6 ml·min^{-1}. Absorption rate in the absence (V) and in the presence (V_D) of 5×10^{-4} M phloridzin, in nmoles·cm^{-1}·min^{-1}. Mediated transport $V_T = V - V_D$. Each value is the mean with its standard error. Number of experiments in parentheses.

Glucose (mM)		Temperature (°C) 37 (Basal)	32	39
2	V	91.2 ± 4.5 (17)	56.2 ± 7 (11)	81.1 ± 4 (7)
	V_D	40.8 ± 3.3 (16)	24.1 ± 4 (8)	37.4 ± 4 (8)
	V_T	50.4	32.1	43.7
5	V	205.3 ± 15.9 (17)	163.5 ± 22 (9)	164 ± 11 (8)
	V_D	108.5 ± 8.9 (12)	102.1 ± 6 (7)	76 ± 16 (8)
	V_T	96.8	61.4	88
10	V	447.2 ± 34.9 (20)	339.1 ± 26 (9)	356 ± 30 (7)
	V_D	275.7 ± 26.2 (13)	216.5 ± 30 (6)	213.4 ± 22 (8)
	V_T	171.5	122.6	142.6
20	V	654.7 ± 30.9 (9)	524.2 ± 64 (4)	540 ± 37 (3)
	V_D	418.7 ± 32.8 (7)	265.0 ± 10 (3)	298 ± 20 (3)
	V_T	236	259.2	242

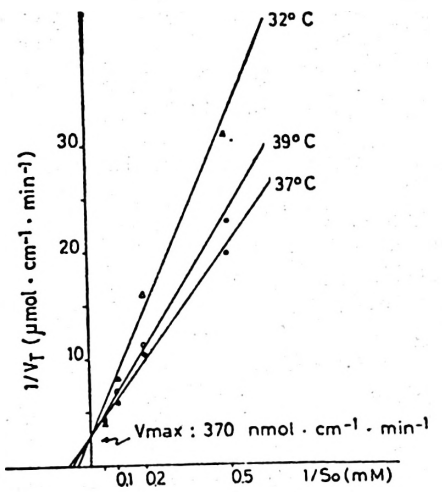

Fig. 1. *Lineweaver-Burk plotts for glucose transport in rat at different temperatures.*

Apparent values of Michaelis constant, K'_m, calculated from these lines, were 14.8 (39° C), 13.1 (37° C) and 21.2 mM (32° C). Thus a clear increase in K'_m results when the temperature of the solution decreases from 37° to 32° C.

From the V_D values at different temperatures, the apparent mass-transfer coefficient for the passive component, K'_D, was estimated as 25.5 and 23.3 nmol $\cdot cm^{-1} \cdot min^{-1} \cdot mM^{-1}$ at 37° and 32° respectively.

Discussion

The influence of the perfusion solution temperature on the kinetics of glucose intestinal absorption is negligible between 37° and 39° C whereas it is clearly manifested between 37° and 32° C. As the temperature changes from 32° to 37° C, total absorption increases as well as the passive absorption in the presence of phloridzin and the non-passive mediated transport. As a consequence, the apparent value of mass transfer coefficient K'_D, corresponding to the passive component, rises with the temperature, while K'_m for the mediated transport of sugar decreases. These changes were to be expected, from the well known dependence of diffusion and enzymatic reactions on the temperature.

However, a correct interpretation of the results must take also into consideration the passive diffusion of sugar through the unstirred layers. The data supplied by GLADDEN and ZOLE (3) for diffusion of glucose in water, allow to estimate that the free diffusion coefficient D at low glucose concentrations changes from 7.9 to 8.8×10^{-6} $cm^2 \cdot sec^{-1}$ as temperature increases from 32° to 37° C, an increment of more than 11 %. Moreover the possibility of changes in the effective thickness of the unstirred layers, δ, has not to be discarded. If at 32° C, D becomes lower and δ higher than at 37° C an important change in the unstirred water layers resistance would have to be expected. A temperature increase must, of course, raise the true masstransfer coefficient K'_D for the transepithelial passive component. As the rate of the non-passive component V_T also increases with non appreciable modification of the saturation rate V_{max}, it is suggested that the true K_m for the mediated transport has to be higher at 37° C than at 32° C. These two factors would co-operate in reaching the steady state of the absorption process at a lower sugar concentration at the epithelial surface, S_m, when the solution is at 37° C rather than at 32° C.

Glucose diffusion across the unstirred layers can be expressed by $D/\delta\ (S_o - S_m)$, $S_o - S_m$ being the difference in concentration between the bulk solution (S_o) and that at the aqueous-membrane interface (S_m). Therefore, by passing from 32° to 37° C D and $S_o - S_m$ will increase while δ might decrease; the diffusion rate, thus, will increase facilitating the whole process of glucose intestinal absorption.

Other factors may be involved in the results, e.g., possible changes in blood

circulation affecting glucose elimination from the subepithelial space. The present results are evidently not enough to evaluate the quantitative importance of all the mechanisms affected by varying temperatures and which may be partly responsible for the observed changes in glucose absorption. They are enough however to show the complexity of the problem and to emphasize the significance of the unstirred water layers in studies of intestinal absorption kinetics.

Resumen

Se estudia la cinética de la absorción intestinal por yeyuno de rata a diferentes temperaturas de la solución de perfusión *in vivo*. El componente no pasivo (transporte activo) se mide como diferencia entre la absorción control y la absorción en presencia de florricina 5×10^{-4} M. Los resultados a 37° y 39° C no difieren significativamente, pero a 32° C hay una clara disminución en la absorción total, así como en los componentes pasivo y activo. Si la temperatura aumenta de 32° a 37° C el valor aparente de K_m para el transporte de glucosa disminuye (de 21,2 mM a 13,1 mM), mientras que la V_{max} no cambia y el coeficiente aparente de transferencia de masa aumenta ligeramente. Los resultados se discuten tomando en consideración el efecto de la temperatura en la difusión a través de las capas de agua no agitadas y en los procesos de transporte activo.

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