VARIATION IN D-GLUCOSE UPTAKE ALONG THE INTESTINAL TRACT

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(Received February 12, 1987)

The variation in D-glucose uptake along the intestinal tract was examined by dividing the entire rat small intestine into 10 segments of equal length and by using everted sacs. The superiority of jejunum to ileum in D-glucose uptake, which is generally accepted, was supported by this work. It was attributed mainly to the variation in the maximal transport velocity of the Michaelis-Menten type carrier-mediated transport system with a clear kinetic border in the distal location of the small intestine, between segments 7 and 8. Information obtained in this study is useful for understanding and estimating the absorption process, or the time-course of absorption, of D-glucose as it passes down in the intestinal tract.

Keywords — intestinal D-glucose uptake; regional variation; carrier-mediated transport; kinetic gradient; unstirred water layer

INTRODUCTION

It has been reported that various compounds are preferentially absorbed by carrier-mediated transport systems of the Michaelis-Menten type, *e.g.*, bile acids,¹⁾ amino acids,²⁾ vitamins³⁾ and sugars,^{4–8)} and that the absorption capacity for a substrate varies along the intestinal tract; that is to say, there exists a kinetic gradient or lack of homogeneity of carrier systems. The entire intestinal tract should be studied in detail for more precise understanding of the variation in the intestinal absorption processes of these compounds as they pass down the intestinal tract. However, most studies are restricted to comparison between the roughly divided regions, for example, comparison between jejunum and ileum. Thus, it is not clear to what extent the absorption capacity varies in each region and where the kinetic border is, if it does exist. We examined these aspects for D-glucose by using everted sacs in this study as a part of a project in which effects of variation in the absorption capacity along the intestinal tract and intestinal transit on the intestinal drug absorption were investigated.

MATERIALS AND METHODS

Chemicals — D-[U-¹⁴C]Glucose (14.4 mCi/mmol), L-[l-¹⁴C]glucose (47.0 mCi/mmol) and

[G-³H]inulin (430 mCi/g) were purchased from New England Nuclear (Boston, MA). D-Glucose (Tokyo Kasei Kogyo Co., Tokyo), L-glucose (Sigma Chemical Co., St. Louis, MO) and other reagents were commercially obtained and were of analytical quality.

Methods — Male Wistar rats weighing 300–350 g were used without fasting prior to experiments. The preparation of everted gut sacs and the procedure of uptake experiments were as previously described.⁹⁾ Briefly, 2.5-cm length sacs were incubated for 0.5 min at 37 °C and 100 cycles/min in 20 ml of Krebs-Ringer bicarbonate buffer (pH 7.4) with D-glucose and inulin as the marker for the adherent mucosal fluid. Required D-glucose concentrations were achieved by amounts of unlabeled D-glucose. Tracer amounts of D-[U-¹⁴C]glucose and [G-³H]inulin were used for quantification. The isoosmolality (290 mosmol/kg) was maintained by changing NaCl concentration. After incubation the wet tissue weight and the amount of D-glucose taken up into the tissue were determined. The uptake rates were expressed in terms of the nanomoles of the substrate taken up per 100 mg wet weight of tissue per min (nmol/100 mg/min). A direct proportionality between the uptake and time was maintained. It should also be noted that a cy-

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lindrical geometry was maintained by a substrate free buffer in the serosal side, supporting a cylindrical model of everted sac for data analysis. L-Glucose was used for estimation of passive transport.

The entire small intestine, from the pylorus to the ileocecal junction, was equally divided into 10 segments and numbered from the most proximal segment. The uptake rates of D-glucose were determined in each segment and those of L-glucose in segment 5.

Data Analyses—Kinetic analyses were carried out as follows: we assumed, for comparison with our *in situ* perfusion experiments, that everted sacs are right cylinders with smooth surfaces, we also considered the effects of the unstirred water layer (UWL) because they may not be negligible, even in everted sacs, in the uptake of such highly membrane permeable substance as D-glucose. The expression of the uptake rate for a Michaelis-Menten type carrier-mediated transport with a simultaneous passive transport in the presence of UWL and on the sink condition in the tissue, which was originally derived by Winne,¹⁰⁾ was slightly modified as follows:

$$\text{uptake rate} = \frac{SD}{\delta/10000} \{ C_b + F - (F^2 + qK_m C_b)^{1/2} \} \times 1000 \times 60 \quad (1)$$

where

$$q = \frac{1}{1 + (\delta/10000) P_m / D}$$

$$F = \frac{1}{2} q \left\{ \frac{K_m}{q} + \frac{(\delta/10000)(V_{\max}/1000)}{D} - C_b \right\}$$

$$S = 2\pi R (100/W)$$

where C_b (mM) is the bulk phase concentration in the medium; D is the diffusion constant, $7.0 \times 10^{-6} \text{ cm}^2/\text{s}$ for D-glucose;⁹⁾ K_m (mM), $V_{\max}$ (nmol/s/cm²) and P_m (cm/s) are the Michaelis constant, the maximal transport velocity and the membrane permeability coefficient, respectively. The effective thickness of UWL (δ) of 104 μm was taken from the report by Dugas *et al.*,¹¹⁾ in which the effects of UWL in everted gut sacs were studied by changing the shaking rate. The S is the outer surface area of the intestinal cylinder

corresponding to 100 mg of the wet tissue weight. The absorbing surface corresponds to the outer surface of the cylinder in the everted sacs. The values of the wet tissue weight per unit length of the intestine (W) and the outer radius of the intestinal cylinder (R) used for calculation were 139 mg/cm and 0.28 cm, respectively, obtained from our previous perfusion study.⁹⁾ In the presence of the passive transport alone the uptake rate is expressed as follows:

$$\text{uptake rate} = SqP_m C_b \times 1000 \times 60 \quad (2)$$

In segment 5, the values of K_m , $V_{\max}$ and P_m were determined by simultaneously fitting Eq. 1 to the data of D-glucose and Eq. 2 to the data of L-glucose by a non-linear regression method, MULTI.¹²⁾ In other segments, the values of K_m and $V_{\max}$ were determined by fitting Eq. 1 to the data of D-glucose, using the value of P_m obtained in segment 5 as a known parameter.

RESULTS AND DISCUSSION

D-Glucose uptake in every segment exhibited concentration dependent saturation kinetics of

FIG. 1. Uptake Rate versus Concentration in Medium of D-Glucose in Segment 1 (Closed Square), Segment 5 (Closed circle) and Segment 10 (Closed Triangle) and of L-Glucose in Segment 5 (Open Circle)

The entire rat small intestine was equally divided into 10 segments and numbered from the most proximal segment. Curves correspond to the parameters obtained by non-linear least square fitting as described in the text. Obtained parameters are shown in Fig. 2 and in its figure legend. Each point and vertical bar represents the mean $\pm$ S.E. of three experiments, using everted sacs from different rats.

FIG. 2. The Michaelis Constant (K_m) and the Maximal Transport Velocity (V_{max}) along the Intestinal Tract, Obtained by Non-linear Least Square Fitting as Described in the Text

The entire rat small intestine was equally divided into 10 segments and numbered from the most proximal segment. The value of the membrane permeability coefficient (P_m) was $(1.1 \pm 0.1) \times 10^{-5}$ cm/s. Each point and vertical bar represents the fitted value of the parameter and the S.D., respectively.

the Michaelis-Menten type carrier-mediated transport component with a passive transport component. Figure 1 shows the uptake rate *versus* concentrations of D-glucose in some representative segments and that of L-glucose in segment 5. D-Glucose uptake was greatest in the jejunum represented by segment 5 and poorest in the distal ileum represented by segment 10. D-Glucose uptake in the duodenum (segment 1) was slightly smaller than in segment 5. On the other hand, the uptake of L-glucose in segment 5 was much smaller than that of D-glucose in the same segment and in the distal ileum where D-glucose uptake was the poorest. The uptake exhibited concentration independent linear kinetics, corresponding to the uptake by the passive transport alone. Thus it appeared that in every segment the passive transport component is minor and can be represented by the uptake of L-

glucose in segment 5.

The values of K_m and V_{max} along the intestinal tract are shown in Fig. 2 and the value of P_m was $(1.1 \pm 0.1) \times 10^{-5}$ cm/s. The variation in the values of V_{max} was found to be critical as a determinant of the superiority of jejunum to ileum in D-glucose uptake, while that of K_m was found to be less significant throughout the intestinal tract, especially in the region from segments 3 to 10 which consists of the ileum and most of the jejunum. Thus it appeared that the variation in the uptake of D-glucose along the intestinal tract is attributable to the variation in the quantity or the number of qualitatively homogeneous carriers. It should also be noticed that a clear kinetic border with variation in values of V_{max} was found in the distal location of the small intestine, between segments 7 and 8.

The superiority of jejunum to ileum in D-glucose uptake has been extensively demonstrated in various animal species. For example, in rat brush border membrane vesicles more prominent overshoot phenomenon has been demonstrated in the jejunum than in the ileum.^{5,6} Rider *et al.* has reported that although the jejunum is superior to the ileum in absorption capacity as a whole, the Michaelis constant, as well as, the maximal transport velocity is much larger in the jejunum than in the ileum of rats by perfusion experiments.⁴ However, their values of the Michaelis constant are considered to be greatly overestimated due to extensive effects of UWL in the perfusion systems as demonstrated in our previous report.⁹ Their value of the Michaelis constant in the jejunum (29 mM) is one order larger than that obtained in this study (3.3 mM in the segment 5) and those obtained in other studies with rats in which the effects of UWL were estimated or greatly reduced by vigorous shaking or stirring in *in vitro* experimental techniques, *e.g.*, 1.7 mM by Richter *et al.*,¹³ 2.2 mM by Thomson¹⁴ and 6.2 mM by Karasov and Diamond.¹⁵ In the same way, the larger Michaelis constant in jejunum than in ileum in their study is at least partly attributable to the effects of UWL, too. Because the effects of UWL become more prominent as absorption capacity increases, even if the value of the true Michaelis constant in the jejunum is equal to that in the ileum as in this study, the apparent value would be larger in the jejunum if the jejunum had larger absorption capacity than the

ileum and if the effects of UWL were not taken into account. Using human brush border membrane vesicles, Harig *et al.* has recently reported that the uptake of D-glucose occurred in the order of distal jejunum > proximal jejunum > distal ileum,⁷⁾ and these results are similar to ours. However, they concluded that at least 3 different transport systems for D-glucose absorption exist in the small intestine because of the variation in the values of the Michaelis constant which conflicts with our results.

Factors which may contribute to the absorption variation are metabolism of D-glucose and variation in the surface area along the intestinal tract. However, it has been reported that nearly 100% of ¹⁴C-labeled D-glucose taken up by the intestinal tissue was recovered as D-glucose by thin-layer chromatography, suggesting that metabolism of D-glucose in the intestinal tissue is negligible.¹³⁾ As for surface area, the variation in the effective absorption surface area as well as the anatomy along the intestinal tract should be considered in order to discuss D-glucose uptake mechanism in detail in comparison with results in brush border membrane vesicles. However, at present it is difficult to discuss the mechanism because of lack of information as to effective absorption surface area.

In jejunum, it has already been demonstrated that the values of K_m , V_{max} and P_m obtained in our studies using everted sacs are compatible with those obtained in our perfusion studies in which the effects of UWL were taken into consideration.⁹⁾ Therefore, it is expected that the parameters obtained in this study are applicable to more physiological conditions, namely, to the perfused intestine and then, possibly, to *in vivo*. Thus information obtained in this study are considered useful for understanding and estimating the intestinal absorption process, or the time-course of absorption, of D-glucose as it passes down in the intestinal tract.

Acknowledgement This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan.

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