Kinetic Characterization of Carrier-Mediated Transport Systems for D-Glucose and Taurocholate in the Everted Sacs of the Rat Colon

Shigemitsu TOMEI,^a Mayumi TORIMOTO,^a Yayoi HAYASHI,^a Katsuhisa INOUE,^a Hiroaki YUASA,^{*,a} and Jun WATANABE^b

^a Graduate School of Pharmaceutical Sciences, Nagoya City University; 3-1 Tanabe-dori, Mizuho-ku, Nagoya 467-8603, Japan; and ^b College of Pharmacy, Nihon University; 7-7-1 Narashinodai, Funabashi, Chiba 274-8555, Japan.

Received December 16, 2002; accepted March 7, 2003

The present study was aimed at kinetically characterizing the carrier-mediated transport systems for D-glucose and taurocholate in the rat colon, compared with their respective counterparts in the small intestine. The transport of these compounds was evaluated by measuring the initial uptake into everted intestinal tissue sacs. The uptake of both D-glucose and taurocholate was highly saturable, conforming to Michaelis-Menten kinetics without an appreciable nonsaturable transport component. The Michaelis constant (K_m) was 0.43 and 0.021 mM, respectively, for D-glucose and taurocholate and the maximum transport rate (J_{max}) was 0.82 and 0.056 nmol/min/100 mg wet tissue weight (wtw), respectively. For both compounds, these values of K_m and J_{max} in the colon were one to three orders of magnitude smaller than those in the small intestine, suggesting that the transport systems in the colon have by far a higher affinity and a lower transport capacity than their counterparts in the small intestine. However, it is now evident from kinetic studies that carrier-mediated transport systems for D-glucose and taurocholate are also present in the colon. It will be interesting to explore the possibility that they could be used for oral drug delivery *via* the colon. Their physiological roles would also be of interest.

Key words D-glucose; taurocholate; carrier-mediated transport; colon; rat

The colon has so far attracted much less attention than the small intestine as a site of drug absorption and carrier-mediated drug transport in the colon has not been a subject for extensive investigation. However, our recent studies have revealed that the colonic absorption of riboflavin is mediated by a carrier similar to one in the small intestine.^{1,2)} Furthermore, we recently suggested that Na⁺-dependent carrier-mediated transport systems for D-glucose and taurocholate may be present in the rat colon.³⁾ The intestinal carriers of D-glucose and taurocholate have now been cloned in the human and also in the rat, and are reported to be also expressed in the colon, although at levels lower than those in the small intestine.^{4,5)} However, the functional levels of those carriers have not been clarified yet. Taking into account that recent advances in controlled-release techniques have allowed the delivery of drugs to the lower part of the gastrointestinal tract following oral administration and raised the general interest in colonic drug absorption, it is of interest to further characterize those transport systems and explore the possibility that they could be used for oral drug delivery *via* the colon. Potential strategies for that purpose would include, if identical or similar carriers are available in the colon and small intestine, designing drugs targeted to such a carrier (or carriers) and as well absorbed from the colon as from the small intestine. Such drugs would be suitable for a sustained-release formulation that is effective even after reaching the colon.

MATERIALS AND METHODS

Chemicals D-[¹⁴C(U)]Glucose (9.3 GBq/mmol), [³H(G)]-taurocholic acid (74 GBq/mmol), [1,2-¹⁴C]polyethylene glycol (PEG) 4000 (0.481 GBq/g) and [1,2-³H]PEG 4000 (0.069 GBq/g) were purchased from PerkinElmer Life Sciences, Inc. (Boston, MA, U.S.A.). Unlabeled D-glucose and taurocholic acid were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Soluene-350, a tissue solubilizer, and

Scintisol EX-H, a scintillation fluid, were purchased from Packard Instrument Co., Inc. (Meriden, CT, U.S.A.) and Dojindo Lab. (Kumamoto, Japan), respectively. All other reagents were of analytical grade and commercially obtained.

Uptake Experiments Uptake experiments were conducted using everted sacs (2 cm in length) prepared from the colon of male Wistar rats, weighing about 300 g and unfasted, as previously reported.^{6,7)} Briefly, test solutions were prepared in Krebs-Ringer-bicarbonate buffer (KRB: 118 mM NaCl, 4.7 mM KCl, 2.5 mM CaCl₂, 1.2 mM KH₂PO₄, 1.2 mM MgSO₄, 25 mM NaHCO₃ and oxygenated with 95%O₂–5%CO₂ gas, pH 7.4). For the tests, trace amounts of [¹⁴C]D-glucose and [³H]PEG 4000 as a nonabsorbable marker, or [³H]taurocholate and [¹⁴C]PEG 4000 were added to the solutions. The concentrations of D-glucose and taurocholate were adjusted by adding unlabeled compounds. Everted sacs were preincubated for 5 min in substrate-free buffer before the initiation of uptake by incubation of everted sacs in 20 ml of a test solution at a temperature of 37 °C and a shaking rate of 100 strokes/min. Uptake was terminated by rinsing the everted sacs briefly in ice-cold saline. The tissue uptake was evaluated by determining the radioactivity after solubilization of the everted sac, using 1 ml Soluene-350 as a tissue solubilizer and 5 ml Scintisol EX-H as a scintillation fluid.

Data Treatment The uptake was estimated by subtracting the amount in the adherent fluid and that initially adsorbed to the everted sac from the total amount associated with the tissue sample, and was expressed in terms of 100 mg wet tissue weight (wtw). The adherent fluid volume was estimated by dividing the amount of PEG 4000 associated with the everted sac by its concentration in the medium. The initial uptake was evaluated at 5 min, as the uptakes of D-glucose and taurocholate were previously shown to increase in proportion to time at least up to 5 min.³⁾ The uptake clearance (CL_{up}) was calculated by dividing the uptake rate in the initial 5-min phase by the concentration in the medium (C_m).

* To whom correspondence should be addressed. e-mail: yuasa@phar.nagoya-cu.ac.jp

The expression of CL_{up} for Michaelis–Menten type carrier-mediated transport is as follows:

$$CL_{up} = \frac{J_{max}}{K_m + C_m} \quad (1)$$

where J_{max} and K_m are the maximum transport rate and the Michaelis constant, respectively. The kinetic parameters of J_{max} and K_m were estimated by fitting Eq. 1 to the experimental data of CL_{up} versus C_m profiles, using a nonlinear regression program, WinNonlin (Pharsight Co., Mountain View, CA, U.S.A.), and the reciprocal of the variance as the weight.

RESULTS AND DISCUSSION

The uptake clearance of D-glucose (Fig. 1A) and taurocholate (Fig. 1B) decreased with concentration, demonstrating the involvement of saturable transport, and reached a negligible level, suggesting that nonsaturable transport by passive diffusion is negligible. Accordingly, kinetic analyses revealed that the uptake of both D-glucose and taurocholate conformed to the model of a single Michaelis–Menten type carrier-mediated transport component (Eq. 1). The kinetic parameters for carrier-mediated transport of D-glucose and taurocholate in the colon are summarized in Table 1, together with those in the small intestine from our earlier study.⁶⁾

For D-glucose transport, K_m was smaller in the colon by a factor of about 18 than in the small intestine and so was J_{max} by a factor of about 260 and, accordingly, so was J_{max}/K_m by a factor of about 14 (Table 1). Thus, the carrier-mediated D-glucose transport system in the colon was found to be kinetically distinct, having a much higher affinity and a lower capacity, compared with its counterpart in the small intestine. However, the D-glucose carrier in the colon seemed to be an

isoform of SGLTs (sodium-dependent glucose transporters) which include SGLT1 that has been suggested to be responsible for D-glucose transport in the small intestine. This was because we also found that D-glucose uptake in the colon was Na^+ -dependent³⁾ and completely inhibited by phlorizin (1 mM), a specific inhibitor of SGLTs (data not shown). Since the mRNA of SGLT1 is reportedly present in the rat colon at a trace level (far lower than that in the small intestine),⁴⁾ it is possible that the trace level of SGLT1 might be functionally modulated in a different manner in the colon compared with that in the small intestine. Alternatively, an unidentified SGLT isoform might be responsible for D-glucose transport in the colon. Although two other SGLT isoforms, SGLTs 2 and 3, have so far been identified, both of them are carriers with a lower affinity than SGLT1 and less likely to be involved in D-glucose transport in the colon.⁸⁾ Although the mRNA of human SGLT2, the major isoform in the kidneys, is reportedly expressed at a trace level in the small intestine,⁸⁾ the mRNA of rat SGLT2 has not so far been documented to be present in the intestine, including the colon. SGLT3 has not been identified in the rat, although it has been identified in humans and pigs, and the mRNA of pig SGLT3 is reportedly present in the small intestine.⁹⁾

Also for taurocholate, both K_m and J_{max} were far smaller in the colon by a factor of about 67 and 480, respectively, than in the small intestine (Table 1), demonstrating the kinetic distinctness of the transport system in the former compared with that in the latter. The Na^+ -dependent carrier of ASBT (apical sodium-dependent bile acid transporter) has been suggested to be responsible for the transport of taurocholate in the small intestine (ileum)¹⁰⁾ and its mRNA is reportedly expressed in the rat colon, although only at a trace level (a level far lower than that in the small intestine).⁵⁾ Since tauro-

Fig. 1. Concentration-Dependent Uptake of D-Glucose (A) and Taurocholate (B) by Everted Sacs of Rat Colon

Results are represented as the mean $\pm$ S.E. ($n=3$). The uptake was measured at 37 °C and 5 min. The J_{max} (nmol/min/100 mg wtw) was 0.82 ± 0.08 and 0.056 ± 0.004 , respectively, for D-glucose and taurocholate, and the K_m (mM) was 0.43 ± 0.08 and 0.021 ± 0.002 , respectively, as the computer-fitted parameter with S.E.

Table 1. Parameters of Carrier-Mediated Transport in Rat Intestinal Everted Sacs: Comparison between the Small Intestine and Colon

		J_{max} (nmol/min/100 mg wtw)	K_m (mM)	J_{max}/K_m (μ l/min/100 mg wtw)
D-Glucose	Small intestine ^{a)}	210	7.8	27
	Colon	0.82	0.43	1.9
Taurocholate	Small intestine ^{a)}	27	1.4	19
	Colon	0.056	0.021	2.7
Riboflavin	Small intestine ^{b)}	4.30×10^{-3}	0.57×10^{-3}	7.5
	Colon ^{b)}	0.74×10^{-3}	0.13×10^{-3}	5.7

Values represent computer-fitted parameters: J_{max} , maximum uptake rate; K_m , Michaelis constant; a) data from Yuasa *et al.*⁶⁾; b) Tomei *et al.*²⁾

cholate transport in the colon was also found to be Na^+ -dependent in our preceding study,³⁾ the trace level of ASBT might be functionally modulated in the colon in a different manner to that in the small intestine, or an unidentified ASBT isoform or some other class of carrier might be responsible for taurocholate transport in the colon, like the above speculation about the D-glucose carrier.

Including carrier-mediated transport systems for riboflavin examined in our previous study (Table 1), we consistently found ones in the colon with a higher affinity and a lower capacity than their respective counterparts in the small intestine. It seems reasonable to find ones with a higher affinity in the colon, the distal part of the gastrointestinal tract where substrate concentrations should be lower. These carrier-mediated transport systems in the colon may assist in the efficient uptake of low levels of nutrients, not absorbed in the small intestine but managing to reach the colon. It should be noted that the difference in kinetic characteristics between the small intestine and colon is less profound for riboflavin transport systems than others. In particular, the J_{max}/K_m , which represents the transport efficiency at concentrations far below K_m , for riboflavin in the colon was comparable with that in the small intestine, while those for D-glucose and taurocholate were an order of magnitude smaller in the colon than in the small intestine.

In conclusion, we were successful in obtaining a kinetic characterization of the carrier-mediated transport systems for D-glucose and taurocholate in the rat colon, although the transport systems in the colon, including the one for riboflavin examined in our previous study, were found to have

a higher affinity and a lower transport capacity than their respective counterparts in the small intestine. Similar transport systems may be also present in the human colon. Although this will require evaluation in the human as well as more detailed transport studies in the rat, it is of interest to further explore the possibility that those transport systems could be used for oral drug delivery *via* the colon. Their physiological roles would also be of interest.

Acknowledgment This work was supported in part by a Grant-in-Aid for Scientific Research (C) from Japan Society for the Promotion of Science (#12672155).

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