Glycerol Absorption by Na⁺-Dependent Carrier-Mediated Transport in the Closed Loop of the Rat Small Intestine

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The absorption of glycerol was examined using the closed loop of the rat small intestine *in situ* to clarify the transport mechanism. The absorption of glycerol, evaluated by its disappearance from the intestinal lumen, was saturable and reduced under the Na⁺-free conditions, suggesting the involvement of an Na⁺-dependent carrier-mediated transport system. Furthermore, glycerol absorption was selectively inhibited by several alcohols, among which 1,3-propanediol caused the greatest inhibition, and also by glycerol-3-phosphate and voglibose, which are alcohol-related compounds analogous to glycerol. Several other compounds that did not inhibit glycerol absorption included D-glucose and L-ascorbate, which are known to be transported by specific carriers. Therefore, the carriers for these two compounds do not seem to be involved in glycerol absorption. It is likely that the carrier-mediated transport system involved in glycerol absorption is specific to glycerol and, possibly, some analogous compounds with hydroxyl groups. Thus, the present study has provided *in situ* evidence for the presence of an Na⁺-dependent carrier-mediated transport system for glycerol in the rat small intestine. It would be interesting to examine the possibility that the carrier-mediated glycerol transport system could be involved in drug absorption and also that it could be used for oral drug delivery.

Key words glycerol; carrier-mediated transport; intestine; absorption; rat

It is generally assumed for hydrophilic solutes, which cannot readily cross the cellular lipid membrane, that passive (diffusive) transport *via* the paracellular route is the principal transport mechanism for intestinal absorption unless carrier-mediated transport systems are available. Glycerol is one such hydrophilic solute and it is believed to be absorbed mainly by paracellular passive transport. However, we recently found that glycerol absorption is saturable in the rat small intestine *in situ*,¹⁾ an indication of the potential involvement of carrier-mediated transport. We further revealed in another more recent study using the *in vitro* everted sac method that the carrier-mediated glycerol transport is Na⁺-dependent and secondary active.²⁾ It was also suggested in the same study, from the inhibition of glycerol transport by some alcohol-related compounds analogous to glycerol, such as glycerol-3-phosphate, voglibose and chloramphenicol, that the carrier for glycerol is specific to glycerol and some analogous compounds with hydroxyl groups. Considering that the intestinal absorption of glycerol, a potent oral osmotic dehydrating agent for the treatment of glaucoma, is highly efficient³⁾ and that glycerol is a nutritional component that can be liberated from dietary fat (triglycerides) in the intestinal tract,^{4,5)} it is possible that a carrier-mediated transport system is involved in the efficient absorption and utilization of this nutritional substance. Therefore, to further confirm the suggestion from our earlier studies and clarify the physiological relevance, we examined the absorption of glycerol using the closed loop of the rat small intestine *in situ*.

MATERIALS AND METHODS

Chemicals [¹⁴C(U)]Glycerol (5.28 GBq/mmol) and [1,2-³H]polyethylene glycol (PEG) 4000 (0.056 GBq/g) were purchased from PerkinElmer Life Sciences, Inc. (Boston, MA, U.S.A.). Scintisol EX-H, a scintillation fluid, was purchased from Dojindo Lab. (Kumamoto, Japan). Voglibose was a gift

from Takeda Chemical Industries, Ltd. (Osaka, Japan). All other reagents were of analytical grade and commercially available.

Absorption from the Intestinal Loop Male Wistar rats, weighing about 300 g, were used after fasting overnight. Each rat was anesthetized with urethane (1.125 g/kg, i.p.) and the absorption of glycerol was evaluated as described previously.⁶⁾ Briefly, the abdomen of each rat was cut open, and a 5-cm closed loop was prepared by ligating both ends of the selected segment after internally washing the small intestine (midgut) with saline about 20 cm below the duodeno-jejunal flexure. Solutions were prepared by adding [¹⁴C]glycerol (2 μM) and a trace amount of [³H]PEG 4000 as a nonabsorbable marker to phosphate buffer (20.1 mM Na₂HPO₄·12H₂O, 47.0 mM KH₂PO₄, 101.0 mM NaCl, pH 6.4) and, when necessary, modified as indicated to examine the effects of inhibitors and altered conditions. After introducing 0.5 ml solution, the intestinal loop was returned to the abdominal cavity, and the abdomen was closed by sutures. The rectal temperature was monitored and maintained at 37 °C, using a heat lamp, and then 10 min later the loop was isolated to remove the luminal solution. A portion of the luminal solution (50 μl) was placed in a counting vial and mixed with 3 ml Scintisol EX-H to determine the radioactivity in a liquid scintillation counter (LSC-5100, Aloka Co., Tokyo, Japan). The fraction absorbed was estimated as the fraction that disappeared from the loop after correcting for a volume change based on the change in PEG 4000 concentration.

Statistical Analysis Statistical differences were assessed using analysis of variance (ANOVA) followed by Dunnett's test.

RESULTS AND DISCUSSION

We first examined the concentration- and Na⁺-dependence of glycerol absorption (Table 1). The fraction of the glycerol

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Table 1. Concentration- and Na⁺-Dependence of Glycerol Absorption in the Closed Loop of the Rat Small Intestine

Condition	Fraction absorbed (%)
Control	75.0 ± 1.4
Glycerol (40 mM)	52.1 ± 2.5 ^{a)}
Na ⁺ free ^{b)}	45.2 ± 1.9 ^{a)}

Data represent the mean ± S.E. (*n* = 3). The absorption of [¹⁴C]glycerol (2 μM) was evaluated 10 min after administration. *a)* Significantly different from the control at *p* < 0.05; *b)* substitution of Na⁺ by K⁺.

dose absorbed was significantly reduced at the higher concentration of 40 mM unlabeled glycerol, demonstrating the saturability of glycerol absorption as documented in our previous report.¹⁾ In addition to that, we found in the present study that glycerol absorption was significantly reduced under Na⁺-free conditions, where Na⁺ in the administered solution was substituted by K⁺. These results in the closed loop of the rat small intestine *in situ* are consistent with our earlier finding of Na⁺-dependent and saturable glycerol uptake, with a Michaelis constant of 0.77 mM, in a study using *in vitro* everted sacs²⁾ and this suggests that the Na⁺-dependent carrier-mediated transport system for glycerol also operates *in situ*.

The absorption of glycerol was significantly inhibited by 1,2-propanediol, 1,3-propanediol, 1,2-butanediol, 1,3-butanediol and 1,4-butanediol, as shown in Fig. 1. The inhibition by 1,3-propanediol was the greatest (about 40%), followed by 1,2-propanediol and 1,3-butanediol (about 30%). These results are in general agreement with those in our earlier study using *in vitro* everted sacs.²⁾ In that study, although the inhibition of glycerol uptake by any alcohol did not reach a statistically significant level, 1,3-propanediol caused the greatest inhibition (about 40%) and 1,2-propanediol and 1,3-butanediol caused the second greatest inhibition (about 30%). Among other alcohol-related compounds, glycerol-3-phosphate and voglibose were found to inhibit glycerol absorption significantly, while mephenesin did not (Fig. 2). These results are also in agreement with those obtained using everted sacs,²⁾ although, as an exception, chloramphenicol, an effective inhibitor of glycerol uptake in everted sacs, failed to show a significant inhibitory effect. D-Glucose⁷⁾ and L-ascorbate,⁸⁾ which are known to be transported by specific carriers, were confirmed not to inhibit glycerol transport as in everted sacs. Therefore, the carriers for these two compounds do not seem to be involved in glycerol absorption. Thus, the profile of the inhibitory effect of various compounds on glycerol absorption *in situ* are in general agreement with those *in vitro* and it is likely that the carrier-mediated transport system involved in glycerol absorption is specific to glycerol and, possibly, some analogous compounds with hydroxyl groups.

Voglibose is known to be practically unabsorbable from the small intestine and acts as an antidiabetic agent in the small intestine by reducing the absorption of D-glucose by inhibiting the (α-glucosidase-mediated hydrolytic production of D-glucose from disaccharides,⁹⁾ even although it inhibits glycerol uptake competitively in everted sacs.²⁾ Therefore, this drug seems to be an interesting example of a competitive inhibitor that cannot be transported. It should also be noted that voglibose (molecular weight, 267.3) cannot interfere

Fig. 1. Effect of Alcohols on Glycerol Absorption in the Closed Loop of the Rat Small Intestine

Data represent the mean ± S.E. (*n* = 3). The absorption of [¹⁴C]glycerol (2 μM) was evaluated 10 min after administration in the presence of an alcohol (5 mM) or in its absence. *a)* Significantly different from the control at *p* < 0.05.

Fig. 2. Effect of Various Compounds on Glycerol Absorption in the Closed Loop of the Rat Small Intestine

Data represent the mean ± S.E. (*n* = 3). The absorption of [¹⁴C]glycerol (2 μM) was evaluated 10 min after administration in the presence or absence of a test compound. *a)* Significantly different from the control at *p* < 0.05.

with glycerol absorption at clinical doses (typically 0.2 mg), because its concentration would be 3.7 μM, when taken with (or dissolved in) 200 ml water, or lower in the intestine, which is much lower than its inhibition constant of 3.39 mM estimated in everted sacs.²⁾

A series of water channel proteins have recently been cloned and named aquaporins (AQPs). It is known that a group of AQPs (AQP3,¹⁰⁾ AQP7,^{11,12)} AQP9^{13,14)} and AQP10¹⁵⁾ act as channels also for small neutral solutes, typically glycerol and urea, and these are called aquaglyceroporins. However, permeation through channels is expected to be a linear (unsaturable) process, as reported for glycerol uptake *via* AQP3.¹⁰⁾ It is kinetically distinct from the saturable nature of intestinal glycerol transport. It is also unlikely that such channels require Na⁺ for operation. Therefore, it is unlikely that these aquaglyceroporins would be involved in the carrier-mediated glycerol transport, although we cannot exclude the possibility that they might be functionally heterogeneous and act as glycerol carriers.

The utilization of glycerol by intestinal bacteria might also be involved in the disappearance of glycerol from the intestinal lumen *in vivo*. However, since the intestinal lumen was washed thoroughly to remove its contents, including bacteria, in the present study, it is very unlikely that significant degradation of glycerol occurs in the buffered solution filling the lumen during the absorption experiments.

In conclusion, the present study has successfully provided *in situ* evidence for the presence of an Na⁺-dependent car-

rier-mediated transport system for glycerol in the rat small intestine. It could be a novel transport system specific to glycerol and some analogous compounds with hydroxyl groups. The functional characteristics of the transport system *in situ* are generally similar to those in *in vitro* everted sacs, suggesting the physiological relevance of our findings. Although the molecular entity of the carrier has not been identified, and the structural requirements of substrates remain to be fully described, it would be interesting to examine the possibility that such a carrier-mediated glycerol transport system could be involved in drug absorption and also that it could be used for oral drug delivery.

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