Saturable Absorption of Glycerol in the Rat Intestine

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The permeability of glycerol, a small hydrophilic solute, across the intestinal membrane would be low, if passive diffusion restricted to the paracellular route is the principal transport mechanism as generally assumed for this class of solutes. However, in the present study using a closed loop of rat small intestine *in situ*, we found that the absorption of glycerol was faster than that of urea, a probe solute widely assumed to permeate exclusively *via* the paracellular route. This finding is inconsistent with the paracellular permeation hypothesis, which predicts that the absorption of glycerol, which is larger than urea in terms of molecular size, could not be faster than that of urea. We also found that glycerol absorption was saturable. These findings suggest the involvement of carrier-mediated transport in intestinal glycerol absorption. Glycerol absorption in the colon was also saturable, suggesting the involvement of carrier-mediated transport, although it was much slower than that in the small intestine. Carrier-mediated glycerol transport might play an important role in absorbing glycerol liberated from dietary triglyceride. It would be interesting to further examine the possibility that a carrier-mediated glycerol transport system (or systems) might be involved in drug absorption and also that it might be utilized for oral drug delivery.

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

It is generally assumed for hydrophilic solutes, which cannot readily cross the cellular lipid membrane, that passive diffusion *via* the paracellular route would be the principal transport mechanism for intestinal absorption unless carrier-mediated transport systems are available. The paracellular permeability, which is restricted by the paracellular pore size, is expected to decrease with an increase in molecular size. Glycerol is one of a number of small hydrophilic solutes for which the permeability has been shown to conform to the paracellular permeation hypothesis in a series of *in vitro* studies using excised tissues from the rat jejunum and colon.^{1,2} However, an earlier *in vitro* study by Rubin and Deren showed saturable glycerol uptake by excised tissue of the rabbit small intestine,³ an indication of the potential involvement of carrier-mediated transport. It should be noted that glycerol is known to be rapidly absorbed from the small intestine and orally effective as a potent osmotic dehydrating agent for the treatment of glaucoma.⁴ Glycerol is also a nutritional component of dietary fat (triglycerides).⁵ Although most of the dietary glycerol is known to be absorbed as a constituent of monoglycerides after partial hydrolysis of triglycerides, a significant portion (about 20%) has been suggested to be absorbed as free glycerol liberated from monoglycerides by further hydrolysis.⁶ Considering these aspects, the possibility that carrier-mediated transport might be involved in the efficient absorption and utilization of this nutritional substance cannot be excluded. Thus, until now, the intestinal transport mechanism of glycerol has remained unconfirmed.

We report here our recent findings in the rat intestine *in situ* that suggest the involvement of carrier-mediated transport in glycerol absorption. This study should help guide future studies to clarify the intestinal transport mechanism of glycerol.

MATERIALS AND METHODS

Chemicals [¹⁴C(U)]Glycerol (5.28 GBq/mmol), [¹⁴C]Urea (2.12 GBq/mmol) and [1,2-³H]polyethylene glycol (PEG) 4000 (55.5 MBq/g) were purchased from PerkinElmer Life Sciences, Inc. (Boston, MA, U.S.A.). Unlabeled glycerol and urea were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan) and Cahc Co., Ltd. (Nagoya, Japan). All other reagents were of analytical grade and commercially obtained.

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 or urea was evaluated as described previously.⁷ 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 with saline, either in the small intestine (midgut) at about 20 cm below the duodeno-jejunal flexure or in the colon immediately below the ileo-cecal junction. Solutions containing glycerol or urea were prepared by adding trace amounts of ¹⁴C-labeled glycerol or urea to phosphate buffer (20.1 mM Na₂HPO₄·12H₂O, 47.0 mM KH₂PO₄, 101.0 mM NaCl, pH 6.4). Unlabeled glycerol or urea was added to adjust the concentration and a trace amount of [³H]PEG 4000 was added as a nonabsorbable marker. 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 half an hour 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 5 ml Scintisol EX-H (Dojindo Lab., Kumamoto, Japan), a scintillation fluid, to determine the radioactivity in a liquid scintillation counter (LSC-5100, Aloka Co., Tokyo, Japan). The fraction absorbed (*F_a*) was estimated as the fraction that disappeared from the loop after correcting

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for a volume change based on the change in PEG 4000 concentration. Assuming first-order absorption, the absorption rate constant (k_a) was estimated as follows:

$$k_a = -\frac{\ln(1-F_a)}{t} \quad (1)$$

where t represents the absorption (experimental) period. The apparent membrane permeability clearance (CL_{app}) was estimated as the product of k_a and the luminal volume ($100 \mu\text{l}/\text{cm}$).

Statistical Analysis Levels of statistical significance were assessed using Student's t -test or, when multiple comparisons were needed, analysis of variance (ANOVA) followed by Dunnett's test.

RESULTS AND DISCUSSION

Saturable Glycerol Absorption in the Small Intestine

The fraction of the dose absorbed (F_a) of glycerol was as much as 92.1% over 30 min at the lowest concentration of 0.002 mM ($2 \mu\text{M}$) in the small intestine, as shown in Table 1. The apparent membrane permeability clearance (CL_{app}) was $8.55 \mu\text{l}/\text{min}/\text{cm}$. The F_a was unchanged when the concentration was increased to 1 mM, but reduced to 73.1% when the concentration was further increased to 40 mM. Thus, glycerol absorption was found to be dependent on concentration (saturable). It was surprising to find, in addition, that the absorption of glycerol was faster than that of urea at all three concentrations, ranging from 0.002 to 40 mM. On the other hand, the F_a of urea was almost constant (about 60%) over the wide range of concentrations, suggesting a passive transport mechanism. Urea is a small hydrophilic solute with a molecular weight (MW) of 60 and generally believed to be transported by passive diffusion exclusively *via* the paracellular route. If glycerol (MW , 92) is also transported exclusively by paracellular passive diffusion, the absorption of glycerol, which is

larger in terms of molecular size, could not be faster than that of urea, because permeation should be in principle restricted by the paracellular pore size. Therefore, these findings suggest that glycerol transport is elevated, compared with what can be expected from a passive (diffusive) mechanism, and saturable due to the significant involvement of carrier-mediated transport. Because the transcellular passive permeability of glycerol, if involved, can be assumed to be comparable with that of urea, as discussed in detail in a later section, the total passive permeability of glycerol *via* the paracellular and transcellular routes would not be greater than that of urea (CL_{app} of about $3 \mu\text{l}/\text{min}/\text{cm}$). Therefore, at the lowest concentration of 0.002 mM, carrier-mediated transport may account for more than 60% of glycerol transport with a CL_{app} of about $8.55 \mu\text{l}/\text{min}/\text{cm}$.

If there is water absorption, paracellular permeation could be enhanced due to an additional solute flux associated with water flux (solvent drag effect). On the other hand, water secretion could reduce paracellular permeation by hindering diffusive flux. We therefore evaluated water movement, using the final-to-initial concentration ratios (C_f'/C_i') of PEG 4000 (a nonabsorbable marker). As shown in Table 1, moderate water secretion was persistently suggested by the fact that the C_f'/C_i' values were smaller than unity (dilution of PEG 4000). However, the C_f'/C_i' values did not significantly change in each series of experiments using different concentrations for glycerol and urea. Thus, the concentration-dependence of glycerol absorption cannot be explained by the effect of water secretion. Water secretion tended to be slightly greater for glycerol than for urea, as indicated by the slightly smaller C_f'/C_i' values obtained experimentally. This could be a factor that might reduce the permeation of glycerol relative to that of urea, but this does not explain our finding of faster absorption for glycerol.

Saturable Glycerol Absorption in the Colon We also examined glycerol absorption in the colon (Table 2). The ab-

Table 1. Absorption of Glycerol and Urea from the Closed Loop of the Rat Small Intestine

Substance	Concentration (mM)	F_a (%)	CL_{app} ($\mu\text{l}/\text{min}/\text{cm}$)	C_f'/C_i'
Glycerol	0.002	92.1 ± 1.1	8.55 ± 0.51	0.774 ± 0.070
	1	90.1 ± 1.2	7.75 ± 0.41	0.745 ± 0.158
	40	73.1 ± 0.2^a	4.38 ± 0.03^a	0.733 ± 0.105
Urea	0.002	60.9 ± 1.9^c	3.13 ± 0.16^c	0.873 ± 0.026
	1	62.3 ± 0.6^c	3.25 ± 0.05^c	0.836 ± 0.025
	40	64.9 ± 2.4^b	3.51 ± 0.23^b	0.841 ± 0.034

Data are represented as the mean $\pm$ S.E. ($n=3$). *a*) Significantly different from the value for 0.002 mM at $p<0.05$ (ANOVA). *b*) Significantly different from the value for glycerol at the same concentration at $p<0.05$; *c*) $p<0.01$ (t -test). F_a , fraction absorbed in 30 min; CL_{app} , apparent membrane permeability clearance; C_f'/C_i' , final-to-initial concentration ratio of PEG 4000 (nonabsorbable marker).

Table 2. Absorption of Glycerol and Urea from the Closed Loop of the Rat Colon

Substance	Concentration (mM)	F_a (%)	CL_{app} ($\mu\text{l}/\text{min}/\text{cm}$)	C_f'/C_i'
Glycerol	0.002	15.1 ± 0.7	0.55 ± 0.03	0.901 ± 0.049
	1	6.3 ± 0.4^a	0.22 ± 0.02^a	0.877 ± 0.022
	40	2.5 ± 0.9^a	0.08 ± 0.03^a	0.783 ± 0.019
Urea	0.002	10.0 ± 0.3^c	0.35 ± 0.01^c	0.888 ± 0.081
	1	11.4 ± 1.4^b	0.40 ± 0.05^b	1.052 ± 0.129
	40	9.1 ± 1.6^b	0.32 ± 0.06^b	0.978 ± 0.061

Data are represented as the mean $\pm$ S.E. ($n=3$). *a*) Significantly different from the value for 0.002 mM at $p<0.05$ (ANOVA). *b*) Significantly different from the value for glycerol at the same concentration at $p<0.05$; *c*) $p<0.01$ (t -test). F_a , fraction absorbed in 30 min; CL_{app} , apparent membrane permeability clearance; C_f'/C_i' , final-to-initial concentration ratio of PEG 4000 (nonabsorbable marker).

sorption of glycerol was saturable and, at the lowest concentration of 0.002 mM, faster than that of urea, although the F_a values were much smaller than those in the small intestine. The degree of water secretion suggested by the C'_t/C'_i values was comparable or less than that in the small intestine, was not significantly changed at different concentrations in each series of experiments with glycerol and urea, and was not significantly different between experiments with glycerol and urea, particularly at the lowest concentration. These results are similar to those in the small intestine and suggest that carrier-mediated transport might also be involved in glycerol absorption in the colon, although the transport capability appeared to be much lower in the colon than in the small intestine. It should be noted that a reduction in F_a was observed at a lower concentration in the colon (1 mM) than in the small intestine (40 mM), suggesting that the carrier-mediated transport system in the colon may have a higher affinity and, hence, may be kinetically distinct from that in the small intestine.

Passive Permeation of Glycerol and Urea Glycerol absorption in the colon was almost negligible with a nominal CL_{app} of 0.08 $\mu\text{L}/\text{min}/\text{cm}$ at the highest concentration of 40 mM (Table 2), suggesting that glycerol simply does not cross the colonic membrane by passive diffusion via any route, paracellular or transcellular. The paracellular route in the colon seemed to be quite tight, almost completely excluding this small solute. Urea absorption in the colon was independent of concentration, suggesting a passive transport mechanism as in the small intestine, and at an appreciable level with a CL_{app} of about 0.35 $\mu\text{L}/\text{min}/\text{cm}$ (Table 2). Assuming that the transcellular passive permeability increases with lipophilicity, which can be represented by the octanol/water partition coefficient ($P_{o/w}$), and diffusivity, which can be represented by the diffusion coefficient that is inversely proportional to the square root of the molecular weight ($MW^{1/2}$),⁸⁾ the $P_{o/w}/MW^{1/2}$ could be an index of the transcellular passive permeability. Using the $P_{o/w}$ values of 0.0025 and 0.0016,⁹⁾ respectively, for glycerol (MW , 92) and urea (MW , 60), the $P_{o/w}/MW^{1/2}$ values were estimated to be comparable for urea (0.00021) and glycerol (0.00026). Therefore, it is likely that the transcellular passive permeability of urea is comparable with that of glycerol and, hence, negligible. Thus, the significant absorption of urea could be almost totally due to paracellular permeation, consistent with the generally accepted assumption.

The area of the anatomical villous surface, which is perfused by blood in the subepithelial capillary network and, hence, represents the absorptive surface area (S_a), is reportedly about 3-fold greater per unit length in the small intestine (6.5 cm^2/cm for midgut) than in the colon (2.2 cm^2/cm).¹⁰⁾ This could lead to the same degree of difference in CL_{app} , which can be described as the product of the apparent permeability coefficient (P_{app}) and S_a ($CL_{app} = P_{app} \cdot S_a$), or in the clearance of each permeation component. However, for both glycerol and urea, it is unlikely that the component of transcellular passive permeability, which was suggested to be negligible in the colon, is elevated in the small intestine to an appreciable level due to the difference in S_a . Therefore, the absorption of urea in the small intestine by passive transport, which was suggested from the concentration-independence (Table 2), could be mainly accounted for by paracellular per-

meation, consistent with the generally accepted assumption. The CL_{app} of urea absorption in the small intestine was greater than that in the colon by a factor of about 10 (Tables 1 and 2), which is much greater than that for S_a . This difference suggests a larger P_{app} in the small intestine due to the looser paracellular route associated with a difference in the density or size distribution of paracellular pores.

Carrier-Mediated Glycerol Transport The intestinal transport mechanism of glycerol has not been investigated extensively, although Rubin and Deren suggested about three decades ago that carrier-mediated transport might be involved in glycerol transport following an uptake study *in vitro* using tissue from the rabbit small intestine.³⁾ They found that glycerol uptake was saturable and inhibited selectively by *N*-ethylmaleimide, but not by glucose, phlorizin and ethylene glycol. Another study by Winne and Remischovsky showed a few years earlier that glycerol absorption was faster than that of urea in the perfused rat small intestine *in situ*, although they did not comment on this.¹¹⁾ However, contradicting this, two more recent *in vitro* studies using excised tissues of the rat jejunum and colon suggested that glycerol permeation across such tissues conforms to the paracellular permeation hypothesis, showing that its permeability is lower than that of urea.^{1,2)} Although the reason for this discrepancy is not clear, our results in the present study support the former two studies suggesting the involvement of carrier-mediated transport in intestinal glycerol transport.

A series of water channel proteins have recently been cloned and named aquaporins (AQPs), and a subfamily of AQPs named aquaglyceroporins (AQP3, AQP7, AQP9 and AQP10) are known to act as channels for small neutral solutes, typically glycerol and/or urea, as well.^{12–17)} Among them, AQP3 is reportedly present in the small intestine and colon of the rat.¹⁸⁾ In humans, AQP9 and AQP10 have so far been found in the small intestine.^{17,19)} However, permeation through channels is expected to be a linear (unsaturable) process, kinetically distinct from the saturable transport found for glycerol. In addition, AQP3 and AQP9 are suggested to be localized at the basolateral membrane,^{18,19)} while it is obvious that, to achieve rapid and saturable glycerol transport as observed in the rat intestine, a carrier-mediated transport system is required at the brush border membrane for the entry of glycerol into epithelial cells. However, the cellular localization of AQP10 has not been reported yet. Thus, it is unlikely that these aquaglyceroporins would be involved in the carrier-mediated glycerol transport systems, although we cannot exclude a possibility that AQP10 or unidentified aquaglyceroporins might be functionally heterogeneous and act as active or facilitated carriers of glycerol.

Conclusion Although more detailed studies are required, it is likely that carrier-mediated transport systems are present in the intestine (small intestine and colon) for the efficient absorption and utilization of glycerol as a nutritional substance. It would be interesting to examine the possibility that such carrier-mediated glycerol transport system(s) might be involved in drug absorption and also that they might be used for oral drug delivery.

REFERENCES

- 1) Sawada T., Ogawa T., Tomita M., Hayashi M., Awazu S., *Pharm. Res.*,

- 8, 1365—1371 (1991).
- 2) Tomita M., Sawada T., Ogawa T., Ouchi H., Hayashi M., Awazu S., *Pharm. Res.*, **9**, 648—653 (1992).
- 3) Rubin A. W., Deren J. J., *Gastroenterology*, **66**, 378—383 (1974).
- 4) Frank M. S. B., Nahata M. C., Hilty M. D., *Pharmacotherapy*, **1**, 147—160 (1981).
- 5) Brisson D., Vohl M.-C., St-Pierre J., Hudson T. J., Gaudet D., *BioEssays*, **23**, 534—542 (2001).
- 6) Mattson F. H., Volpenhein R. A., *J. Biol. Chem.*, **239**, 2772—2777 (1964).
- 7) Yuasa H., Kuno C., Watanabe J., *J. Pharm. Pharmacol.*, **49**, 26—29 (1997).
- 8) Ballard B. E., Nelson E., *J. Pharmacol. Exp. Ther.*, **135**, 120—127 (1962).
- 9) Leo A., Hansch C., Elkins D., *Chem. Rev.*, **71**, 525—616 (1971).
- 10) Permezel N. C., Webling D. D' A., *J. Anat.*, **108**, 295—296 (1971).
- 11) Winne D., Remischovsky J., *J. Pharm. Pharmacol.*, **22**, 640—641 (1970).
- 12) Echevarria M., Windhager E. E., Frindt G., *J. Biol. Chem.*, **271**, 25079—25082 (1996).
- 13) Ishibashi K., Kuwahara M., Gu Y., Kageyama Y., Tohsaka A., Suzuki F., Marumo F., Sasaki S., *J. Biol. Chem.*, **272**, 20782—20786 (1997).
- 14) Kuriyama H., Kawamoto S., Ishida N., Ohno I., Mita S., Matsuzawa Y., Matsubara K., Okubo K., *Biochem. Biophys. Res. Commun.*, **241**, 53—58 (1997).
- 15) Tsukaguchi H., Shayakul C., Berger U. V., Mackenzie B., Devidas S., Guggino W. B., van Hoek A. N., Hediger M. A., *J. Biol. Chem.*, **273**, 24737—24743 (1998).
- 16) Tsukaguchi H., Weremowicz S., Morton C. C., Hediger M. A., *Am. J. Physiol.*, **277**, F685—F696 (1999).
- 17) Ishibashi K., Morinaga T., Kuwahara M., Sasaki S., Masashi I., *Biochim. Biophys. Acta*, **1576**, 335—340 (2002).
- 18) Koyama Y., Yamamoto T., Tani T., Nihei K., Kondo D., Funaki H., Yaoita E., Kawasaki K., Sato N., Hatakeyama K., Kihara I., *Am. J. Physiol.*, **276**, C621—C627 (1999).
- 19) Okada S., Misaka T., Matsumoto I., Watanabe H., Abe K., *FEBS Lett.*, **540**, 157—162 (2003).