D-Glucose Triggers Multidrug Resistance–Associated Protein (MRP)-Mediated Secretion of Fluorescein Across Rat Jejunum *in Vitro*

Igor Legen[1] and Albin Kristl[1,2]

Received September 15, 2003; accepted January 9, 2004

Purpose. To examine the transport characteristics of the multidrug resistance–associated protein (MRP) substrate fluorescein across the isolated rat small intestinal segments.

Methods. The transport of fluorescein was studied in side-by-side diffusion chambers under short-circuited conditions at physiological pH.

Results. The serosal-to-mucosal permeability of fluorescein significantly exceeded the permeability in the opposite direction in the jejunum, but not in the ileum. This asymmetry in transport in the jejunum was observed only when D-glucose was present at the mucosal side of the tissue, and not in the presence of D-galactose or D-mannitol. In the presence of D-glucose at the mucosal side, serosal-to-mucosal permeability of fluorescein in the jejunum can be divided into an active (Michaelis–Menten constant, K_M = 1.07 mM; maximum flux of the substrate, J_{max} = 14.0 nmol/h · cm^2) and a passive component (passive permeability, $P_{pas} = 2.51 \times 10^{-6}$ cm/s). The polarization of fluorescein transport was almost completely abolished by MRP inhibitor, benzbromarone (50 or 100 μM, applied apically), and by MRP/P-glycoprotein inhibitor, verapamil (200 μM, applied apically).

Conclusions. D-glucose at the mucosal side activates fluorescein secretion across rat jejunum by an apical MRP, most probably by isoform 2 (MRP2), which could have an impact on the intestinal absorption of MRP substrates.

KEY WORDS: active secretion; drug absorption; fluorescein; multidrug resistance–associated protein (MRP); side-by-side diffusion cells.

INTRODUCTION

Fluorescein is a well-known marker for evaluating the paracellular permeability of intestinal epithelial cells (1,2). This organic molecule has two net negative charges at physiological pH (3) and is thus believed to cross the epithelial cells only through the tight junctions. However, it has been demonstrated recently that fluorescein can also be used as a specific probe for the multidrug resistance–associated protein (MRP) efflux pump in several cell cultures (4,5). The MRP family currently has nine members (MRP1-9) and is one branch of the ATP-binding cassette (ABC) superfamily of transmembrane proteins that use the energy of ATP hydrolysis to translocate their substrates across biological membranes (6). MRPs are primary organic anion transporters and transport a range of nonconjugated amphipathic organic anions and neutral compounds conjugated to acidic ligands such as glucuronide, glutathione, and sulfate, although some MRPs may act as unconjugated drug/glutathione co-transporters (7).

Six MRP isoforms (MRP1-6) have been identified so far in the small intestine (8), and among them, MRP2 has the best identified role (7). MRP2 localizes to the apical membrane of enterocytes (9) and has been shown to contribute to direct intestinal secretion of 2,4-dinitrophenyl-*S*-glutathione (10), grepafloxacin (11), and the abundant, food-derived carcinogen 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) (12), using Eisai hyperbilirubinmic rats (EHBR, Sprague-Dawley strain) and Groningen yellow transport deficient rats (TR$^-$, Wistar strain) in which MRP2 is hereditarily defective. Furthermore, the absorption of PhIP was 2-fold higher in the TR$^-$ rats than in control rats, which clearly demonstrates that MRP2 reduces the oral bioavailability of this carcinogen (12). It appears that MRP2 acts as a protective barrier in the small intestine and may affect the absorption, not only of organic anions, but also of neutral compounds (13). However, it is not yet clear whether MRP2 plays an important role at typical oral drug dosages because of the limited literature data (14) about the key kinetic parameters for MRP needed to give an indication of saturability (Michaelis–Menten constant, K_M) and capacity (maximum flux, J_{max}) in native intestinal tissues. Additionally, little is known about the functional activity of MRP2 in different regions of the small intestinal tract (11) or about the factors that regulate MRP2 activity in the small intestine.

In the current study, we investigated the basic characteristics of MRP-mediated transport of fluorescein across the isolated rat small intestinal segments using side-by-side diffusion cells. Special stress was laid on the effect of D-glucose at the mucosal side on the intestinal MRP activity.

MATERIALS AND METHODS

Chemicals

Fluorescein sodium, D-mannitol, and (±)-verapamil hydrochloride were obtained from Fluka (Deisenhofen, Germany). Benzbromarone, ketoprofen, amiloride hydrochloride, and ibuprofen were from Sigma (Deisenhofen, Germany). D-glucose and methyl-α-D-glucopyranoside were from Aldrich (Steinheim, Germany), and D-galactose was from Merck (Darmstadt, Germany). All chemicals used in this study were of analytical grade.

Transport Studies

The experiments conform to the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Council of Europe, European Treaty Series-No. 123, Strasbourg, 1986).

Three- to 4-month-old male Wistar rats (250–320 g) were obtained from the Medical Research Center (Ljubljana, Slovenia) and had been fasted for 18 h before the experiment. After rats were killed by CO_2 inhalation, the small intestine was immediately excised and placed into an ice-cold bubbled (carbogen, 95:5 O_2/CO_2) 10 mM solution of D-glucose in Ringer buffer for not longer than 30 min. The jejunum (25 cm distally from the ileocecal junction) and the ileum (25 cm

[1] Faculty of Pharmacy, University of Ljubljana, Aškerčeva 7, 1000 Ljubljana, Slovenia.

[2] To whom correspondence should be addressed. (e-mail: kristla@ffa.uni-lj.si)

0724-8741/04/0400-0635/0 © 2004 Plenum Publishing Corporation

proximal to the ileocecal junction) were used. The intestine was rinsed with ice-cold Ringer buffer to remove luminal contents, cut into 3-cm-long segments, excluding visible Peyer's patches, and opened along the mesenteric border. It was then mounted on a special insert and placed between two Easy-Mount side-by-side diffusion chambers with an exposed tissue area of 1 cm^2 (Physiologic Instruments, San Diego, CA, USA).

During the experiment, the tissue was incubated on both sides with Ringer buffer (pH 7.45) containing 10 mM D-glucose at the serosal and, unless otherwise stated, at the mucosal sides. The incubation medium was kept at 37°C and continuously gassed with carbogen.

After 30 min of equilibration, the substance under investigation (fluorescein, ketoprofen, or ibuprofen) was added to the mucosal or serosal side to study mucosal-to-serosal (m-to-s) or serosal-to-mucosal (s-to-m) transport, respectively. The final volume of the solution in each compartment was 2.5 ml.

To study the effects of different sugars, 10 mM of D-glucose at the mucosal side was replaced with an equimolar concentration of D-mannitol or D-galactose. For studies using inhibitors of MRP (50 or 100 μM benzbromarone), MRP/P-glycoprotein (200 μM verapamil) or Na^+/H^+ exchange (1 mM amiloride), the inhibitor was added 20 min before fluorescein to the mucosal side only and was present in the mucosal solution throughout the experiment. In the experiments examining the effect of lower pH of the incubation buffer, the amounts of HCO_3^-, $H_2PO_4^-$, and HPO_4^{2-} in the original Ringer buffer were changed in order to give pH 6.5.

Samples of 250 μl were withdrawn from the acceptor compartment at 25-min intervals up to 175 min or 100 min (experiments with MRP and MRP/P-glycoprotein inhibitors, respectively) and replaced by the appropriate Ringer buffer solution to maintain constant concentration of sugars and/or inhibitors in the acceptor compartment.

The tissues were short-circuited to zero potential difference (PD) by a multichannel voltage-current clamp (model VCC MC6, Physiologic Instruments, San Diego, CA, USA). Tissue viability and integrity were checked by monitoring PD, short circuit current (I_{sc}), and transepithelial electrical resistance (TEER) every 25 min, and additionally, in the experiments with D-mannitol at the mucosal side, by recording the increase of I_{sc} and PD after the addition of methyl-α-D-glucopyranoside (25 mM) to the mucosal compartment at the end of experiments. The background potential (asymmetry of the electrodes and liquid junction potential) was compensated before mounting the tissue in the diffusion chamber system. Additionally, I_{sc} and TEER were corrected for fluid resistance. TEER was determined according to Ohm's law.

Analytical Procedure

Concentrations of fluorescein were analyzed by fluorescence (λ_{EX} = 485 nm, λ_{EM} = 535 nm) using a microplate reader (Tecan, Salzburg, Austria). The concentrations of ketoprofen and ibuprofen were analyzed by high performance liquid chromatography (HPLC) as described previously (15).

Data Analysis

The apparent permeability coefficients (P_{app}) were calculated from Eq. (1):

$$P_{app} = \frac{dQ}{dt} \cdot \frac{1}{A \cdot C_o} = \frac{J_{tot}}{C_o} \quad (1)$$

where dQ/dt is the steady-state appearance rate on the acceptor side of the tissue, A is the exposed area of the tissue ($1cm^2$), C_o is the initial concentration of the drug in the donor compartment, and J_{tot} is the total measured flux, expressed in the case of s-to-m transport as:

$$J_{tot} = J_{act} + J_{pas} \quad (2)$$

where J_{act} and J_{pas} represent the active and the passive components, which are defined as:

$$J_{act} = \frac{J_{\max} \cdot C_o}{K_M + C_o} \quad (3)$$

and:

$$J_{pas} = P_{pas} \cdot C_o \quad (4)$$

J_{max} is the maximum flux of the substrate, K_M the apparent Michaelis–Menten constant, and P_{pas} the permeability coefficient for the passive transport.

The kinetic parameters of the s-to-m transport of fluorescein were obtained by fitting J_{tot} to Eq. (5) using the nonlinear least-squares regression analysis program Microsoft Excel (Microsoft Corporation, Seattle, USA).

$$J_{tot} = \frac{J_{\max} \cdot C_o}{K_M + C_o} + P_{pas} \cdot C_o \quad (5)$$

Statistics

Results are expressed as means ± SEM. Two-group comparisons were analyzed by unpaired two-tailed t test or paired Student's two-tailed t test. In the case of an unpaired t test, F-test for variances was first applied. If the variances were equal, the standard Student's t test was performed, otherwise Behrens–Fisher test was used. The difference was considered significant if $p < 0.05$.

RESULTS

Effect of Sugars on the Transport Characteristics of Fluorescein Across Rat Jejunum

Fluorescein permeated almost 2-fold faster in the s-to-m direction than in the opposite direction when 10 mM D-glucose was present at the mucosal side of the tissue (Fig. 1A). When D-glucose at the mucosal side was replaced by D-galactose, another metabolizable sugar, the difference between m-to-s and s-to-m permeability became smaller and insignificant ($p > 0.05$) (Fig. 1B), and was completely abolished when only the nonmetabolizable D-mannitol was present at the mucosal side (Fig. 1C). These results show that D-glucose at the mucosal side activates secretion of fluorescein across rat jejunum *in vitro*.

Kinetic Analysis of Fluorescein Fluxes Across Rat Jejunum

The transport characteristics of fluorescein in the s-to-m direction were further analyzed. Figure 2 shows that the total flux of fluorescein can be expressed as the sum of the passive and active components. The kinetic parameters obtained by fitting the total measured flux to Eq. (5) are: $P_{pas} = 2.51 \times 10^{-6}$ cm/s for the passive component and $J_{max} = 14.0$ nmol/h · cm^2 and $K_M = 1.07$ mM for the active component. These

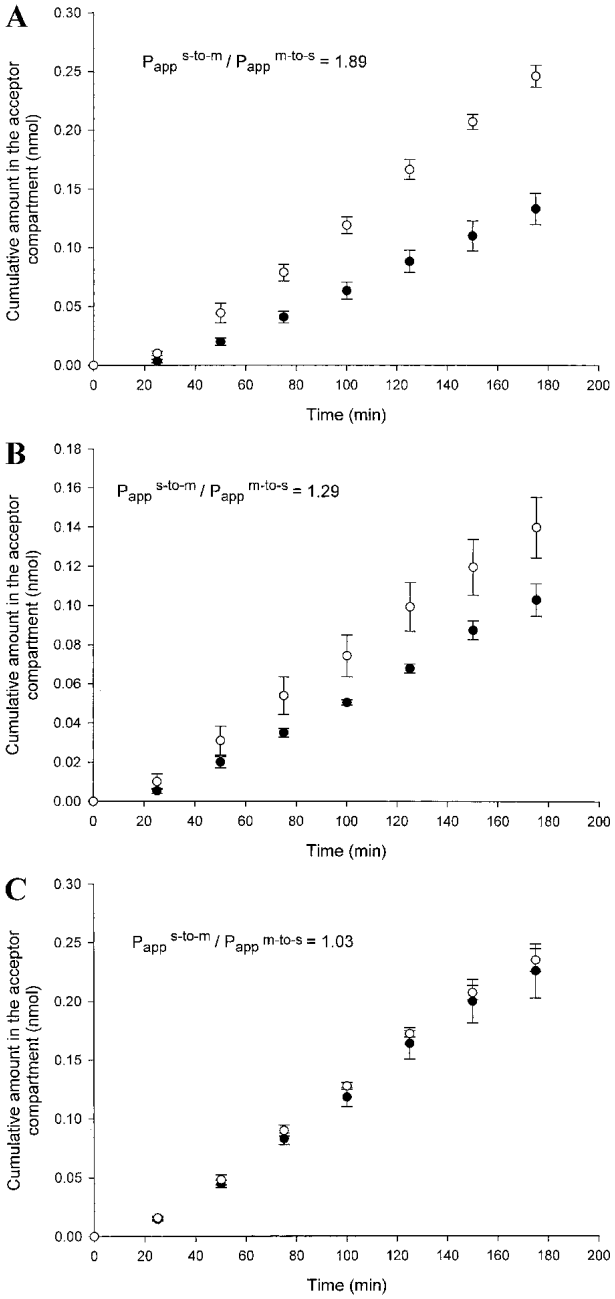

Fig. 1. Effects of (A) 10 mM D-glucose, (B) 10 mM D-galactose, and (C) 10 mM D-mannitol at the mucosal side on the s-to-m (open symbols) and m-to-s (closed symbols) transport of fluorescein (5 μM) across rat jejunum *in vitro*. Ten mM D-glucose was present at the serosal side in all cases. Each point represents the mean ± SEM of three to eight experiments.

results clearly demonstrate the participation of a carrier system in the permeability of fluorescein in the s-to-m direction. On the other hand, the transport of fluorescein in the m-to-s direction exhibits no active component (Fig. 3), however, the average P_{app} value obtained at concentrations ≥500 μM ($P_{app} = 3.63 \times 10^{-6} \pm 0.20 \times 10^{-6}$ cm/s) is higher than the average P_{app} value obtained at lower fluorescein concentrations ($P_{app} = 2.89 \times 10^{-6} \pm 0.44 \times 10^{-6}$ cm/s). This might be a consequence of the reduced back-flux of fluorescein at higher concentrations due to saturation of the active secretion

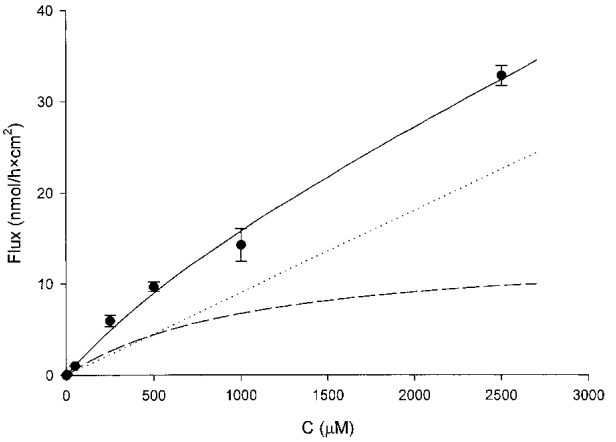

Fig. 2. Concentration dependence of fluorescein transport across rat jejunum in the s-to-m direction. The solid, dashed, and dotted lines represent the fitted total flux, the active and the passive components, respectively, derived from the kinetic parameters that were obtained by fitting the total measured flux to Eq. (5). Each point represents the mean ± SEM of three to six experiments.

in the s-to-m direction, resulting in the higher m-to-s permeability.

Inhibition Studies

Benzbromarone is an MRP inhibitor, probably noncompetitive (16), whereas verapamil is a well-known P-glycoprotein inhibitor (17) that also effectively inhibits MRP transport at concentrations higher than 100 μM (5) and can be classified as an MRP/P-glycoprotein inhibitor. Both benzbromarone and verapamil diminished the polarized transport of fluorescein across rat jejunum (Fig. 4), owing to increased m-to-s and decreased s-to-m transport. Fifty and 100 μM benzbromarone similarly modulated fluorescein transport, with much more pronounced effects on the m-to-s than on the s-to-m permeability.

Regional Difference

The asymmetry of fluorescein transport is much more pronounced in the jejunum than in the ileum (Fig. 5). The net apparent permeability in the ileum is significantly lower than that obtained in the jejunum, mostly because of the pro-

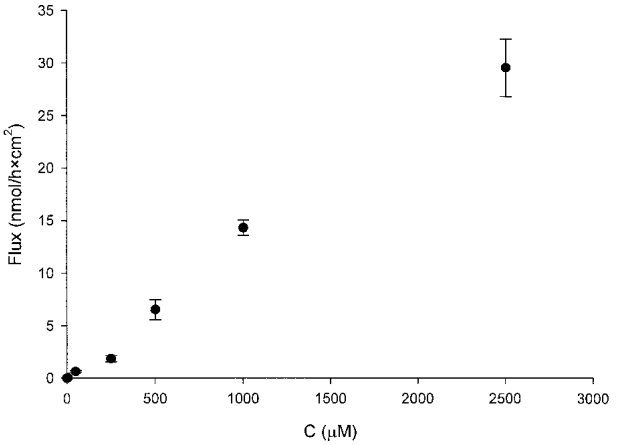

Fig. 3. Concentration dependence of fluorescein transport across rat jejunum in the m-to-s direction. Each point represents the mean ± SEM of three to eight experiments.

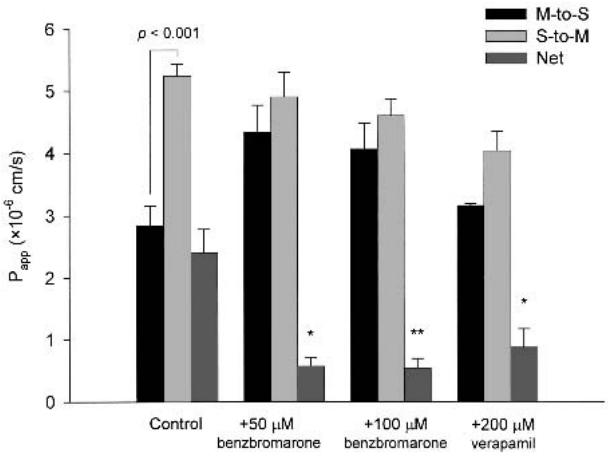

Fig. 4. Influence of MRP (benzbromarone) and MRP/P-glycoprotein (verapamil) inhibitors on the apparent permeability (P_{app}) of fluorescein (5 μM) across the rat jejunum. Net P_{app} was obtained by subtracting the m-to-s permeability from the s-to-m permeability. *p < 0.05, **p < 0.01 significantly different from the net P_{app} obtained in the control experiment (unpaired *t* tests). Data are expressed as the mean ± SEM of three to eight experiments.

nounced decrease in the s-to-m permeability. These results indicate that the carriers that mediate fluorescein efflux are less abundant in the ileum than in the jejunum.

Effect of Amiloride and Low pH of the Incubation Buffer

D-glucose at the mucosal side of the rat jejunum *in vitro* lowers the pH of the mucosal surface microclimate, an effect that can be reduced by the Na^+/H^+ exchange inhibitor amiloride (18). We therefore examined the possibility that D-glucose–induced acidification of the mucosal surface activates active secretion of fluorescein across the rat jejunum. The addition of 1 mM amiloride to the mucosal side did not reduce the secretory-oriented polarization of fluorescein transport, but rather increased it (Table I). In addition, the exclusion of D-glucose from the mucosal side and reducing the incubation buffer pH to 6.5, thus mimicking the D-glucose–induced acidification of the mucosal surface pH (18), markedly increased fluorescein transport in the m-to-s direction and decreased its transport in the s-to-m direction (Table I). These results demonstrate that D-glucose–induced acidification of the mucosal surface does not activate active secretion of fluorescein.

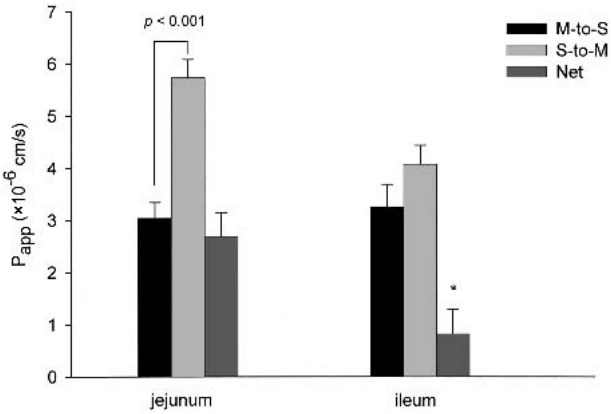

Fig. 5. Regional characteristics of fluorescein (5 μM) transport across rat small intestine. Net P_{app} was obtained by subtracting the m-to-s permeability from the s-to-m permeability. *p < 0.05) significantly different from the net P_{app} obtained in the jejunum (unpaired *t* test). Data are expressed as the mean ± SEM of three to eight experiments.

Table I. The Effects of Amiloride and Lower pH of the Incubation Buffer on the Permeability of Fluorescein (5 μM) Across the Rat Jejunum *In vitro*

Conditions	Apparent permeability, P_{app} ($\times 10^{-6}$ cm/s)		
	m-to-s	s-to-m	$P_{app}^{\text{s-to-m}}/P_{app}^{\text{m-to-s}}$
Control*	3.03 ± 0.30†	5.73 ± 0.35	1.89 ± 0.33
+Amiloride‡	2.35 ± 0.21†	5.60 ± 0.51	2.38 ± 0.47
pH 6.5§	8.62 ± 0.98†	3.21 ± 0.37	0.37 ± 0.09

* The pH of the incubation buffer was 7.45; 10 mM D-glucose was present at the mucosal side of the tissue.
† Significantly different from the corresponding P_{app} value for the s-to-m transport ($p < 0.01$). Data are expressed as the mean ± SEM of three to eight experiments.
‡ The pH of the incubation buffer was 7.45; 10 mM D-glucose and 1 mM amiloride were present at the mucosal side.
§ The pH of the incubation buffer was 6.5; 10 mM D-mannitol was present at the mucosal side instead of D-glucose. 10 mM D-glucose was present at the serosal side in all cases.

Effect of D-Glucose on the Transport Characteristics of Ketoprofen and Ibuprofen

The transport of ketoprofen (Fig. 6A) and ibuprofen (Fig. 6B) was significantly higher in the m-to-s than in the s-to-m direction when 10 mM D-mannitol was present at the mucosal side of the rat jejunum. On the other hand, no asymmetry in the transport of either substance was observed when 10 mM D-mannitol at the mucosal side was replaced by 10 mM D-glucose. This was due to an increase in the s-to-m and a decrease in the m-to-s transport. This suggests that D-glucose at the mucosal side activates secretion of these two nonsteroidal anti-inflammatory drugs (NSAIDs) across the rat jejunum *in vitro*.

DISCUSSION

It has been shown that fluorescein can be used as a specific probe for the MRP efflux pump in several cell lines (4,5). At least six members of the MRP family are present in the small intestine tissue (MRP1-6) (8). Among them, MRP1 and MRP3 localize to the basolateral membrane, whereas MRP2 is present in the apical membrane (7,9,19). The precise localization and functions of other MRP isoforms in the small intestine are still unclear (7). The net secretion of fluorescein observed in this study indicates that an apical MRP member, most probably MRP2, mediates fluorescein transport in the s-to-m direction. Additionally, the polarization of fluorescein transport in the jejunum, but not in the ileum (Fig. 5), is consistent with the reports demonstrating that the expression of MRP2 is higher in the jejunum than in the ileum (9,10).

The contribution of another apically located ATP-dependent efflux pump, P-glycoprotein, to fluorescein secretion is less likely. In general, P-glycoprotein substrates are uncharged and/or organic cations at physiological pH (20), whereas fluorescein is in the form of a dianion at physiologi-

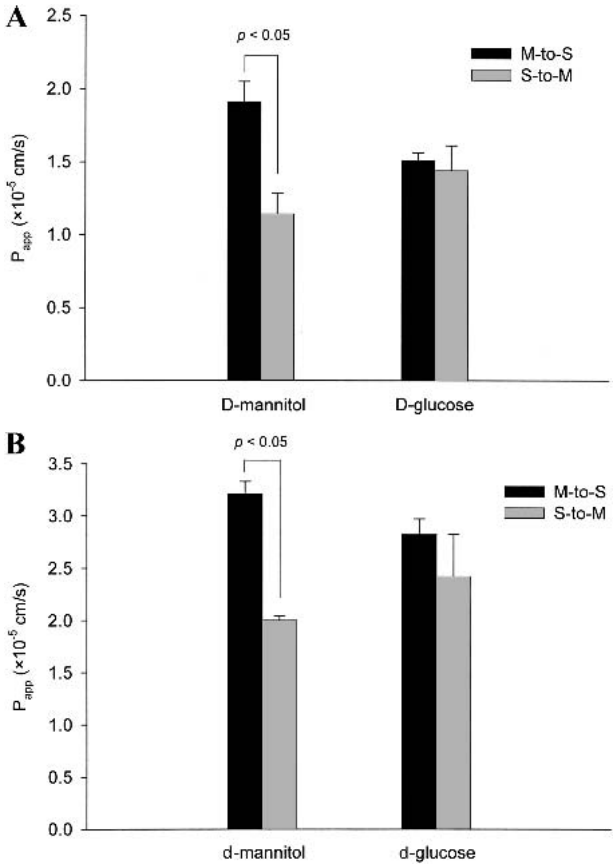

Fig. 6. Effects of 10 mM D-mannitol and 10 mM D-glucose at the mucosal side on the apparent permeability (P_{app}) of (A) ketoprofen and (B) ibuprofen across rat jejunum. Ten mM D-glucose was present at the serosal side in all cases. Data for the experiments with D-mannitol are taken from Ref. 15. Results are expressed as the mean ± SEM of three experiments.

cal pH (3). There are only a few reports that P-glycoprotein can also mediate transport of negatively charged compounds, such as atorvastatin and phenytoin (7,21), albeit at a low rate (7). The net secretion of fluorescein observed in the jejunum, but not in the ileum, is also not in accordance with P-glycoprotein–mediated transport, because P-glycoprotein is much less abundant in the jejunum than in the ileum (22). Additionally, verapamil, which was shown to inhibit both P-glycoprotein (17) and MRP (5), had a similar effect on the fluorescein transport to that of a specific MRP inhibitor, benzbromarone (Fig. 4), indicating that P-glycoprotein is not involved in the secretion of fluorescein across rat jejunum.

The significant asymmetry of the fluorescein transport across rat jejunum was observed only when D-glucose, but not D-mannitol, was present at the mucosal side (Figs. 1A and 1C). The main difference between D-glucose and D-mannitol is that D-glucose can be metabolized, yielding ATP, which is necessary for MRP2-mediated efflux (10), whereas D-mannitol cannot be metabolized. However, the lack of energy substrates does not appear to be the major reason for the diminished net secretion of fluorescein, because D-glucose was present at the serosal side of the intestine in all experiments. Tissue viability during the experiments was assessed by monitoring the tissue electrical parameters. The average PD value of the intestinal segments observed in the experiments with D-mannitol at the mucosal side was −1.18 ± 0.14 mV (n = 6), and increased significantly ($p < 0.05$, paired t test) to −2.18 ± 0.47 mV (n = 6) when methyl-α-D-glucopyranoside, a nonmetabolizable D-glucose analog specific for SGLT1 (Na^+/D-glucose co-transporter), was added to the mucosal side at the end of the experiments. This increased value was not significantly ($p > 0.05$) different from those observed in the experiments with D-glucose [−2.88 ± 0.29 mV (n = 14)] or D-galactose [−2.47 ± 0.31 mV (n = 6)], demonstrating that the tissue functionality during the experiments with D-mannitol was not affected. Additionally, D-galactose at the mucosal side caused a much lower net secretion of fluorescein than D-glucose (Fig. 1B), although both sugars are transported by the same apical (SGLT1) and basolateral (GLUT2) transporters and yield ATP by similar metabolic pathways (23,24). It is likely, therefore, that D-glucose at the mucosal side activates active secretion of fluorescein across rat jejunum, not only by enriching the energy supply, but mostly by another mechanism.

The difference between D-glucose and D-galactose is that D-glucose at the mucosal side causes acidification of the intestinal mucosal surface microclimate (18,25), due to activation of the apical Na^+/H^+ exchanger (26), whereas no such effect is observed for D-galactose (25). However, the lower pH at the mucosal surface caused by D-glucose is probably not responsible for activating fluorescein active secretion since the Na^+/H^+ exchange inhibitor amiloride even increased secretory oriented polarization of fluorescein transport across the rat jejunum (Table I). Additionally, reducing the mucosal surface pH with the lower incubation buffer pH (6.5), without D-glucose at the mucosal side (18), resulted in pronounced absorptive oriented transport of fluorescein across the rat jejunum (Table I), which is in agreement with the mechanism of the absorption of organic acid type drugs from the intestinal tract (15,27). We have previously shown that a pH gradient ($pH_{mucosal\ surface} < pH_{intracellular}$) across the apical membrane of the enterocytes facilitates the absorption of some monocarboxylic drugs across rat jejunum in the m-to-s direction (15,27). This mechanism could also explain the decreased m-to-s permeability of fluorescein caused by amiloride (Table I), as the inhibition of D-glucose induced Na^+/H^+ exchange leads to a decreased pH gradient across the apical membrane of the enterocytes. Therefore, it can be concluded that the lower pH of the mucosal surface does not activate the active secretion of fluorescein, but rather increases its transport in the absorptive direction. However, in the presence of D-glucose at the mucosal side, the active secretion of fluorescein predominates over its absorption. Further studies are needed to elucidate the mechanism by which D-glucose at the mucosal side activates apical MRP activity in the rat jejunum.

Ketoprofen ($pK_a = 4.6$) and ibuprofen ($pK_a = 5.3$) are weak organic acids whose absorption from the gastrointestinal tract is rapid and driven by an inwardly directed proton gradient across the apical membrane of the enterocytes (15,27). As shown in Figs. 6A and 6B, the net absorption of ketoprofen and ibuprofen observed in the experiments with D-mannitol was lower in the presence of D-glucose. Therefore, it appears that D-glucose also activates the secretion of other drugs that are anions at physiological pH, and might modulate their transport *in vivo*.

The kinetic analysis of fluorescein transport in the s-to-m direction (Fig. 2) suggests that the apical MRP being studied

can be classified as a low-affinity [its Michaelis constant (K_M) is in the mM range], high-capacity transporter, in agreement with the observed K_M (>1 mM) for methotrexate and estradiol-17-β-D-glucuronide transport in *Spodoptera frugiperda* ovarian cells expressing MRP2 (16,28). The relatively high K_M of this apical MRP, most probably MRP2, indicates that this transporter might have an impact on the intestinal absorption of organic acid type drugs, at typical oral dosages, because MRP2 is abundantly expressed [even more than multidrug resistance protein 1 (MDR1)/P-glycoprotein] in healthy human jejunum (8).

What could be the physiological relevance of D-glucose induced apical MRP activation? Food may contain not only nutrients, but also potentially harmful substances such as food-derived carcinogens (i.e., PhIP) (12) and toxic organic anions. Activation of the apical MRP efflux pump by the D-glucose that originates from dietary carbohydrates would therefore constitute a defense mechanism against excessive absorption of the xenobiotics recognized by this efflux pump. This might at least partially explain the delayed absorption and/or reduced bioavailability of some organic acid type drugs, such as ketoprofen (29) and furosemide (30), caused by food.

Further, based on the finding that D-glucose at the mucosal side of the rat jejunum triggers active secretion of fluorescein across the intestinal tissue *in vitro*, we suggest that the use of fluorescein as a marker for the paracellular permeability of the jejunum *in vitro* should be limited to experiments excluding D-glucose from the mucosal side. On the other hand, D-glucose at the mucosal side is probably a preferable incubation medium when examining the apical MRP activity in the jejunum *in vitro*.

REFERENCES

1. T. Lindmark, Y. Kimura, and P. Artursson. Absorption enhancement through intracellular regulation of tight junction permeability by medium chain fatty acids in Caco-2 cells. *J. Pharmacol. Exp. Ther.* **284**:362–369 (1998).
2. I. Legen and A. Kristl. Ketoprofen-induced intestinal permeability changes studied in side-by-side diffusion cells. *J. Pharm. Pharmacol.* **54**:1419–1422 (2002).
3. H. Diehl, N. Horchak-Morris, A. J. Hefley, L. F. Munson, and R. Markuszewski. Studies on fluorescein-III. The acid strengths of fluorescein as shown by potentiometric titration. *Talanta* **33**:901–905 (1986).
4. E. V. Betrakova, S. Li, D. W. Miller, and A. V. Kabanov. Pluronic P85 increases permeability of a broad spectrum of drugs in polarized BBMEC and Caco-2 cell monolayers. *Pharm. Res.* **16**:1366–1372 (1999).
5. N. Bandi and U. B. Kompella. Budesonide reduces multidrug resistance-associated protein 1 expression in an airway epithelial cell line (Calu-1). *Eur. J. Pharmacol.* **437**:9–17 (2002).
6. M. Dean, A. Rzhetsky, and R. Allikmets. The human ATP-binding cassete (ABC) transporter superfamily. *Genome Res.* **11**: 1156–1166 (2001).
7. A. H. Schinkel and J. W. Jonker. Mammalian drug efflux transporters of the ATP binding cassette (ABC) family: an overview. *Adv. Drug Deliv. Rev.* **55**:3–29 (2003).
8. J. Taipalensuu, H. Törnblom, G. Lindberg, C. Einarsson, F. Sjöqvist, H. Melhus, P. Garberg, B. Sjöstrom, B. Lundgren, and P. Artursson. Correlation of gene expression of ten drug efflux proteins of the ATP-binding cassette transporter family in normal human jejunum and in human intestinal epithelial Caco-2 cell monolayers. *J. Pharmacol. Exp. Ther.* **299**:164–170 (2001).
9. A. D. Mottino, T. Hoffman, L. Jennes, and M. Vore. Expression and localization of multidrug resistant protein mrp2 in rat small intestine. *J. Pharmacol. Exp. Ther.* **293**:717–723 (2000).
10. Y. Gotoh, H. Suzuki, S. Kinoshita, T. Hirohashi, Y. Kato, and Y. Sugiyama. Involvement of an organic anion transporter (canalicular multispecific organic anion transporter/multidrug resistance-associated protein 2) in gastrointestinal secretion of glutathione conjugates in rats. *J. Pharmacol. Exp. Ther.* **292**:433–439 (2000).
11. K. Naruhashi, I. Tamai, N. Inoue, H. Muraoka, Y. Sai, N. Suzuki, and A. Tsuji. Involvement of multidrug resistance-associated protein 2 in intestinal secretion of grepafloxacin in rats. *Antimicrob. Agents Chemother.* **46**:344–349 (2002).
12. C. G. Dietrich, D. R. De Waart, R. Ottenhoff, I. G. Schoots, and R. P. J. Oude Elferink. Increased bioavailability of the food-derived carcinogen 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine in MRP2-deficient rats. *Mol. Pharmacol.* **59**:974–980 (2001).
13. P. M. Gerk and M. Vore. Regulation of expression of the multidrug resistance-associated protein 2 (MRP2) and its role in drug disposition. *J. Pharmacol. Exp. Ther.* **302**:407–415 (2002).
14. V. D. Makhey, A. Guo, D. A. Norris, P. Hu, J. Yan, and P. J. Sinko. Characterization of the regional intestinal kinetics of drug efflux in rat and human intestine and in Caco-2 cells. *Pharm. Res.* **15**:1160–1167 (1998).
15. I. Legen, S. Žakelj, and A. Kristl. Polarized transport of monocarboxylic acid type drugs across rat jejunum in vitro: the effect of mucolysis and ATP-depletion. *Int. J. Pharm.* **256**:161–166 (2003).
16. É. Bakos, R. Evers, E. Sinkó, A. Váradi, P. Borst, and B. Sarkadi. Interactions of the human multidrug resistance proteins MRP1 and MRP2 with organic anions. *Mol. Pharmacol.* **57**:760–768 (2000).
17. J. S. Woo, C. H. Lee, C. K. Shim, and S.-J. Hwang. Enhanced oral bioavailability of paclitaxel by coadministration of the P-glycoprotein inhibitor KR30031. *Pharm. Res.* **20**:24–30 (2003).
18. I. Legen and A. Kristl. Factors affecting the microclimate pH of the rat jejunum in Ringer bicarbonate buffer. *Biol. Pharm. Bull.* **26**:886–889 (2003).
19. R. A. M. H. Van Aubel, A. Hartog, R. J. M. Bindels, C. H. Van Os, and F. G. M. Russel. Expression and immunolocalization of multidrug resistance protein 2 in rabbit small intestine. *Eur. J. Pharmacol.* **400**:195–198 (2000).
20. Y. Zhang, C. Bachmeier, and D. W. Miller. In vitro and in vivo models for assessing drug efflux transporter activity. *Adv. Drug Deliv. Rev.* **55**:31–51 (2003).
21. X. Wu, L. R. Whitfield, and B. H. Stewart. Atorvastatin transport in the Caco-2 cell model: contributions of P-glycoprotein and the proton-monocarboxylic acid co-transporter. *Pharm. Res.* **17**:209–215 (2000).
22. J. M. Brady, N. J. Cherrington, D. P. Hartley, S. C. Buist, N. Li, and C. D. Klaassen. Tissue distribution and chemical induction of multiple drug resistance genes in rats. *Drug Metab. Dispos.* **30**: 838–844 (2002).
23. H. C. Kutchai. The gastrointestinal system. In R. M. Berne, M. N. Levy, B. M. Koeppen, and B. A. Stanton (eds.), *Physiology*, Mosby, Inc., St. Louis, 1998, pp. 589–674.
24. S. M. Genuth. The endocrine system. In R. M. Berne, M. N. Levy, B. M. Koeppen, and B. A. Stanton (eds.), *Physiology*, Mosby, Inc., St. Louis, 1998, pp. 779–1013.
25. M. Ikuma, H. Hanai, E. Kaneko, H. Hayashi, and T. Hoshi. Effects of aging on the microclimate pH of the rat jejunum. *Biochim. Biophys. Acta* **1280**:19–26 (1996).
26. J. R. Turner, E. D. Black, J. Ward, C. M. Tse, F. A. Uchwat, H. A. Alli, M. Donowitz, J. L. Madara, and J. M. Angle. Transepithelial resistance can be regulated by the intestinal brush-border Na^+/H^+ exchanger NHE3. *Am. J. Physiol. Cell Physiol.* **279**: C1918–C1924 (2000).
27. I. Legen and A. Kristl. pH and energy dependent transport of ketoprofen across rat jejunum in vitro. *Eur. J. Pharm. Biopharm.* **56**:87–94 (2003).
28. A. Bodó, É. Bakos, F. Szeri, A. Váradi, and B. Sarkadi. Differential modulation of the human liver conjugate transporters MRP2 and MRP3 by bile acids and organic anions. *J. Biol. Chem.* **278**:23529–23537 (2003).
29. B. Bannawarth, F. Lapicque, P. Netter, C. Monot, J. N. Tamisier, P. Thomas, and R. J. Royer. The effect of food on the systemic availability of ketoprofen. *Eur. J. Clin. Pharmacol.* **33**:643–645 (1988).
30. J. L. McCrindle, T. C. Li Kam Wa, W. Barron, and L. F. Prescott. Effect of food on the absorption of frusemide and bumetanide in man. *Br. J. Clin. Pharmacol.* **42**:743–746 (1996).