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Immunohistochemical and Functional Characterization of pH-dependent Intestinal Absorption of Weak Organic Acids by the Monocarboxylic Acid Transporter MCT1

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Abstract

The participation of the monocarboxylic acid transporter MCT1 in the intestinal absorption of weak organic acids has been clarified by functional characterization, by use of stably transfected cells, and by immunohistochemical location of the transporter in intestinal tissues.

Immunohistochemical analysis by use of the anti-MCT1 antibody showed that MCT1 is distributed throughout the upper and lower intestines, especially in the basolateral membrane and, to a lesser extent, in the brush-border membrane. When the transporter gene rat MCT1 was transfected into MDA-MB231 cells, transport of benzoic acid, a model weak organic acid that has been generally believed to be transported across the cell membranes by passive diffusion, and lactic acid in rat MCT1-transfected cells was significantly increased compared with transport in cells transfected with the expression vector pRc-CMV alone (mock cells). The observed transport was pH-dependent and activity increased between pH 7·5 and pH 5·5, whereas pH-dependence in mock cells was moderate. Rat MCT1-mediated benzoic acid uptake was saturable, with an apparent Km value of 3·05 mM. In addition, MCT1 increased the efflux of [^{14}C]benzoic acid from the cells. Several weak organic acids were also transported by rat MCT1.

These results show that pH-dependent intestinal absorption of weak organic acids, previously explained in terms of passive diffusion according to the pH–partition hypothesis, is at least partially accounted for by MCT1-mediated transport energized at acidic pH by utilization of the proton gradient as a driving force.

Recent investigations have revealed the importance of membrane transporters in the intestinal absorption, tissue distribution and hepatic and renal elimination of drugs (Tamai & Tsuji 1996; Tsuji & Tamai 1996; Kusuhara et al 1998; Zhang et al 1998). Accordingly, clarification of the mechanisms underlying cell membrane transport is important in understanding drug disposition in the body and for prediction of alterations in the pharmacokinetic behaviour of drugs as a result of interactions with endogenous compounds or co-administered drugs. Membrane transport of weak organic acids such as monocarboxylic acid derivatives has long been believed to occur by passive diffusion according to the pH–partition hypothesis, which predicts that permeability increases with decreasing pH, owing to an increase in the amount of the protonated form of the weak acids (Brodie & Hogben 1957; Nogami & Matsuzawa 1961; Crouthamel et al 1971; Högerle & Winne 1983). However, the characterization of proton- or pH-dependent carrier-mediated transporters such as the proton/oligopeptide transporter PepT1 and others suggested that specialized carrier-mediated membrane transport mechanisms make a significant contribution to the uptake of hydrophilic drugs (Ganapathy & Leibach 1991; Fei et al 1994). We have proposed that participation of pH-dependent, carrier-mediated transport mechanisms can well

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explain the apparent pH-dependent transport observed in membrane physiological studies, and we suggested the involvement of a pH-gradient-dependent proton-monocarboxylic acid cotransporter or bicarbonate-ion-dependent monocarboxylic acid/bicarbonate ion antiporter, or both, in intestinal absorption processes (Simanjuntak et al 1990, 1991; Tsuji et al 1990, 1994; Takanaga et al 1994, 1996; Tamai et al 1995a, 1997). As a molecular candidate for the monocarboxylic acid/bicarbonate ion antiporter, we proposed a member of the anion exchanger family AE2, which is expressed at the brush-border membrane of intestinal epithelial cells (Yabuuchi et al 1998). Endogenous monocarboxylic acids such as lactic acid and pyruvic acid are transported by members of the monocarboxylic acid transporter MCT family in several tissue membranes (Kim et al 1992; Poole & Halestrap 1993; Garcia et al 1994; Price et al 1998). Furthermore, we have demonstrated that monocarboxylic acids such as benzoic acid and salicylic acid are also transported across intestinal epithelial cells by pH-dependent proton cotransporter(s) (Takanaga et al 1994; Tsuji et al 1994; Tamai et al 1995a, 1997), and we suggested the participation of the proton/monocarboxylic acid cotransporter MCT1 (Takanaga et al 1995; Tamai et al 1995b).

MCT1 was originally found as a pH-dependent lactate and pyruvate transporter in Chinese hamster ovary cells and has been shown to be present in a variety of tissues, including the intestine (Poole & Halestrap 1993; Garcia et al 1994; Takanaga et al 1995; Tamai et al 1995b; Price et al 1998). We cloned MCT1 from the rat small intestine and showed that, when expressed in Xenopus oocytes, it transports lactate in the same manner as found in Chinese hamster ovary cells (Takanaga et al 1995). However, it was difficult to examine precisely the functional characteristics of MCT1 for the transport of other monocarboxylic acids in Xenopus oocytes because of high background activity in the cells. In the current study we established a cell line transfected with the rat monocarboxylic acid transporter MCT1, and examined whether it transports exogenous monocarboxylic acids. We also investigated the physiological and pharmacological relevance of the transporter to small-intestinal absorption of several compounds by clarifying the tissue and cellular distribution of the transporter in rats by use of the anti-MCT1 antibody.

Materials and Methods

Materials

L-[^{14}C]Lactic acid (150 mCi mmol^{-1}), [^{14}C]pyruvic acid (7·9 mCi mmol^{-1}) and [^{14}C]mevalonolactone (15 Ci mmol^{-1}) were purchased from American Radiolabelled Chemicals (St Louis, MO). [^{14}C]Benzoic acid (19 mCimmol^{-1}), [^{14}C]butyric acid (15·8 mCi mmol^{-1}), [^{3}H]taurocholic acid (2·0 Ci mmol^{-1}), [^{14}C] *p*-aminohippuric acid (53·1 mCi mmol^{-1}) and $Na_2H^{32}PO_4$ (1 Ci mmol^{-1}) were from New England Nuclear (Boston, MA); [^{14}C]salicylic acid (55 mCi mmol^{-1}), [^{14}C]foscarnet (52 mCi mmol^{-1}), and [^{3}H]methotrexate (30·3 Ci mmol^{-1}) were from Moravec Biochemicals (Brea, CA); [^{14}C]glycylsarcosine (60 mCi mmol^{-1}) was from Amersham International (Buckinghamshire, UK); and [^{14}C]nicotinic acid (38·5 mCi mmol^{-1}) was from Sigma (St Louis, MO). [^{14}C]Faropenem (1·54 MBq mg^{-1}) was kindly supplied by Suntory (Tokyo, Japan). Other chemicals were commercial products of reagent grade.

Immunohistochemical study of MCT1

Two rabbit anti-MCT1 polyclonal antibodies (anti-MCT1/C16 and anti-MCT1/C14) were raised against synthetic peptides (CPQQNSSGDPAEESPV and CQNSSGDPAEEESPV) corresponding to the deduced *C*-terminal amino acid sequences of MEV and MCT1 (Kim et al 1992; Garcia et al 1994), with the addition of an *N*-terminal Cys residue. Each peptide was conjugated to keyhole limpet haemocyanin (Sigma) using Sulpho-SMCC (Pierce, Rockford, IL) as crosslinker, emulsified in an equal volume of Freund's adjuvant (Difco, Detroit, MI), and injected subcutaneously into rabbits. The specific antipeptide antibodies were affinity-purified as described by Carpenter et al (1996).

Immunohistochemical analysis was performed as described elsewhere (Sai et al 1996) with minor modifications. In brief, after fixation of the rats by perfusion with 4% paraformaldehyde, the organs were removed by dissection and further fixed by immersion in the same fixative. They were then frozen and cut into 15-μm sections by means of a cryostat. The sections were first incubated overnight at room temperature with anti-MCT1/C16 or C14 antibody at 1 : 20 dilution. The site of immunoreaction was revealed by successive treatment of the sections with biotinylated anti-rabbit IgG antibody, streptavidin-conjugated horseradish peroxidase and diaminobenzidine-based substrate solution, by means of an LSAB2 kit (Dako). For the negative control, the primary antibody was absorbed by the respective MCT1 peptide at a concentration of 8 μM for 4 h at 4°C before use.

Establishment of rat MCT1-transfected cells

MDA-MB231 cells from ATCC (Rockville, MD) were cultivated at 37°C in Dulbecco's modified

Eagle's medium containing $NaHCO_3$ (44 mM), glutamine (2 mM), penicillin G (100 units mL^{-1}), streptomycin (100 μg mL^{-1}) and 10% foetal calf serum in an atmosphere of 5% CO_2. Rat MCT1 cDNA (Takanaga et al 1995) was subcloned into the pRc-CMV vector (Invitrogen, Carlsbad, CA) between the multicloning sites *Not*I and *Apa*I. After culture for 1 day, MDA-MB231 cells were transfected with pRc-CMV/rat MCT1 using the calcium phosphate precipitation method. As the control, pRc-CMV vector was transfected in the same way. The cells were cultured in the above medium for another 2 days, then the culture medium was changed to the above medium containing 1 mg mL^{-1} G418 for up to 3 weeks and the G418-resistant colonies were isolated. The cells transfected with MCT1-cDNA (rat MCT1) or pRc-CMV vector alone (mock) were used for the measurement of transport activity. Expression of the rat MCT1 protein in the cells was confirmed by Western blotting analysis using anti-MCT1/C16 antibody (data not shown).

Uptake by MDA-MB231 cells transfected with rat MCT1

Uptake of test radiolabelled compounds by MDA-MB231 cells was studied after cultivation of $7-8 \times 10^4$ cells on multiwell dishes (2 cm^2) for 3–4 days in the uptake buffer (NaCl (150 mM), KCl (5 mM), $MgCl_2$ (1 mM), $CaCl_2$ (1 mM), bovine serum albumin (0·2%) and 2-(*N*-morpholino)ethanesulphonic acid (MES; 10 mM), pH 6·0). The cells were pre-incubated for 5 min in the uptake buffer, and the uptake reaction was initiated by incubating the cells in uptake buffer containing radiolabelled compound for a designated time. To terminate uptake the cells were washed three times with ice-cold stop buffer (NaCl (150 mM), KCl (5 mM), $MgCl_2$ (1 mM), $CaCl_2$ (1 mM), phloretin (0·1 mM) and HEPES (2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulphonic acid; 10 mM), pH 7·4). To quantify the radioactivity taken up by the cells, the cells were solubilized with NaOH (1 M, 300 μL), neutralized with HCl (5 M), and transferred to vials for liquid scintillation counting with addition of Cleasol I liquid scintillation cocktail (Nacalai Tesque, Kyoto, Japan). When the effect of pH was to be studied, the pH in the uptake buffer was maintained at the desired value by use of MES or HEPES for acidic or neutral and alkaline pH, respectively. The protein content of the cells was determined by the method of Lowry et al (1951) using bovine serum albumin as the standard.

Data analysis

Most of the results are shown as the cell-to-medium concentration ratio obtained by dividing the amount taken up by the cells by the concentration in the uptake buffer. Kinetic parameters were calculated from the concentration-dependence data by non-linear least-squares analysis using the MULTI programme (Yamaoka et al 1981), by use of equation 1:

$$v = V_{max} \times s/(K_m + s) \quad (1)$$

where v and s are uptake rate and concentration of substrate, respectively, and V_{max} and K_m are maximum uptake rate and Michaelis constant, respectively.

Results

Immunohistochemical location of MCT1 protein along the gastrointestinal tract

The cellular and subcellular location of the transporter along the small intestine is shown in Figure 1. Although MCT1 was detected throughout the gastrointestinal tract from the stomach to the large intestine, only the result in the small intestine is shown. The mucosal epithelium was immunostained with anti-MCT1/C16 (Figure 1A, C, D) and the immunostaining was abolished when the primary antibody was pre-absorbed with the antigen peptide (Figure 1B), confirming the specificity of the immunoreaction. In most immunopositive cells the staining was localized at the cell membrane, with greater intensity on the basolateral membrane than on the apical membrane. Essentially the same results were obtained with anti-MCT1/C14 antibody, so only the results with anti-MCT1/C16 are described. The intensity of epithelial MCT1 immunoreactivity was stronger in more proximal regions of the duodenum–ileum axis, and in lower regions of the crypt–villus axis (Figure 1A). In the relatively immature epithelial cells of crypts and villus bases, the reactivity was localized primarily at the basal membrane and lower lateral membrane (Figure 1C). In contrast, in the absorptive epithelial cells of most villus regions the immunoreactivity was stronger on the upper lateral membrane and strongest beneath the tight junctions, and the brush-border membrane had a distinct but lower-intensity reaction (Figure 1D). Although the data are not shown, the brush-border membrane fraction obtained by the divalent cation precipitation method from rat small intestinal tissues reacted strongly with the antibody, showing that MCT1 protein is present at the brush-border membrane.

The goblet cells and submucosal gland cells were free from immunostaining.

Transport of lactic acid by rat MCT1-transfected MDA-MB231 cells

To confirm the MCT1 transport function in the established MDA-MB231 cells, the stereospecificity and concentration-dependence of lactic acid transport in cells transfected with rat MCT1 or pRc-CMV vector alone were examined. Figure 2A shows the uptake of L-[^{14}C] lactic acid and its stereoisomer by the cells. Uptake of both L- and D-[^{14}C] lactic acids in rat MCT1-transfected cells at tracer concentration were significantly higher than in cells transfected with the pRc-CMV vector alone. Furthermore, the transport activity induced by rat MCT1 was stereospecific with greater activity for the L-isomer. Figure 2B shows the

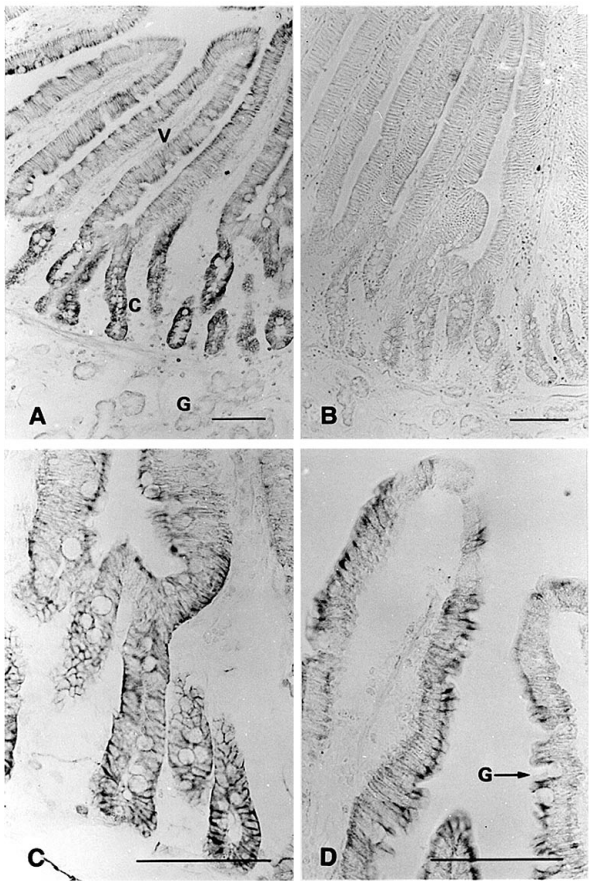

Figure 1. MCT1 immunoreactivity in the small intestine. Sections of A, B. duodenum (× 120) and C, D. jejunum (× 300) were reacted with anti-MCT1/C16 antibody, B. with or A, C, D. without pre-absorption with peptide antigen. A. The immunoreactivity is observed over all the mucosal epithelial cells, with decreasing intensity from the crypts (C) to the tip of the villi (V). No reactivity is observed in submucosal Brunner's glands (G). B. No immunoreaction is obtained with absorbed antibody. C. Magnification of crypts and villus bases. D. Magnification of the upper portions of villi (G shows goblet cells). Scale bars, 100 μm.

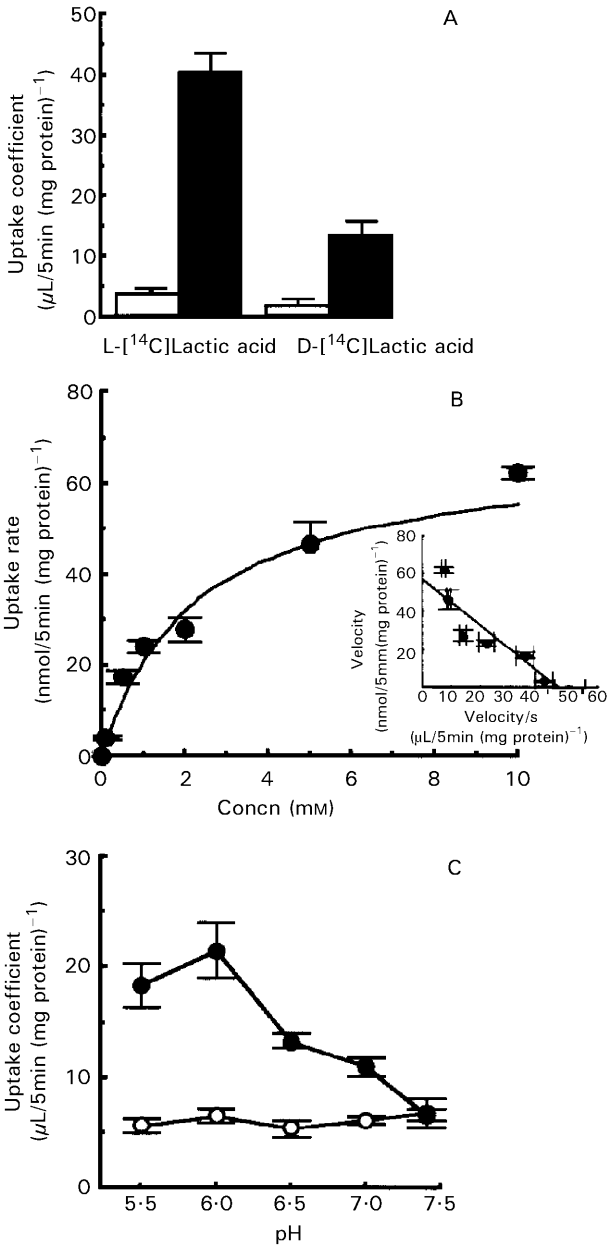

Figure 2. Transport of lactic acid by rat MCT1 expressed in MDA-MB231 cells. Uptake of lactic acid was measured in MDA-MB231 cells transfected with rat MCT1 or pRc-CMV vector alone. Uptake of lactic acid was measured at 4°C for 5 min. A. Transport of L- and D-[^{14}C]lactic acid (3·3 μ M) was measured for cells transfected with rat MCT1 (■) or with pRc-CMV vector alone (□). B. Concentration-dependence of L-lactic acid uptake by rat MCT1 after correction for the uptake by the cells transfected with pRc-CMV vector alone. The inset shows an Eadie–Hofstee plot of the results. C. pH-dependence of L-[^{14}C]lactic acid (3·3 μ M) uptake by rat MCT1 (●) or pRc-CMV vector alone (○). Each result is the means ± s.e.m. from four experiments.

concentration-dependence of uptake of L-lactic acid by rat MCT1 after subtraction of the corresponding uptake by the mock cells. The kinetic parameters estimated by non-linear least-squares regression analysis were K_m $2{\cdot}28 \pm 0{\cdot}47$ mM and V_{max} $68{\cdot}2 \pm 5{\cdot}60$ nmol/5 min (mg protein)$^{-1}$. Furthermore, Figure 2C shows the pH-dependence of L-lactic acid uptake. The uptake of L-[^{14}C]lactic acid increased at lower pH in rat MCT1-transfected MDA-MB231 cells, whereas such an increase at low pH was not observed in mock cells. Accordingly, the cells established in this study were confirmed to contain active monocarboxylic acid transporter MCT1 and were considered suitable for further evaluation of MCT1 function.

Benzoic acid uptake by rat MCT1-transfected cells

Figure 3 shows the time-course of the uptake of [^{14}C]benzoic acid by cells transfected with rat MCT1 or pRc-CMV vector alone. Uptake of [^{14}C]benzoic acid by rat MCT1-transfected cells increased in a time-dependent manner and reached a steady-state in a few minutes, whereas in mock cells the uptake of [^{14}C]benzoic acid was low and did not increase with time. The concentration-dependence of benzoic acid uptake by rat MCT1 was examined and is shown in Figure 4. The estimated K_m and V_{max} values were $3{\cdot}05 \pm 0{\cdot}38$ mM and $168 \pm 14{\cdot}3$ nmol min^{-1} (mg protein)$^{-1}$, respectively. Uptake of [^{14}C]benzoic acid both by rat MCT1-transfected and by mock cells was pH-dependent with increased uptake at lower pH, as shown in Figure 5. The uptake of [^{14}C]benzoic acid by mock cells increased from pH 7·5 to 6·5, but was constant at pH < 6·5. In contrast, uptake of [^{14}C]benzoic acid by rat MCT1-transfected cells increased continuously from pH 7·5 to pH 5·5, distinctly different from the uptake by mock cells.

Benzoic acid efflux in rat MCT1-transfected cells

To examine the direction of benzoic acid transport via rat MCT1, efflux of benzoic acid from cells preloaded with [^{14}C]benzoic acid was studied. The efflux was measured in the efflux medium at pH 6·0, 7·0 and 8·0, and the results are shown in Figure

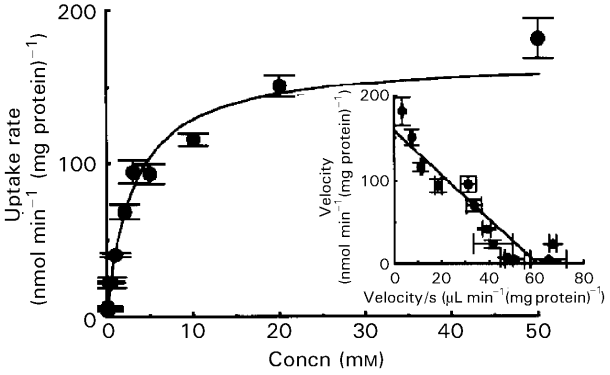

Figure 4. Concentration-dependence of benzoic acid uptake by rat MCT1 expressed in MDA-MB231 cells. Uptake of benzoic acid was measured for 1 min at 37°C and pH 6·0. The result are means ± s.e.m. from three or four experiments after correction for uptake by cells transfected with pRc-CMV vector alone. The inset shows an Eadie–Hofstee plot of the uptake.

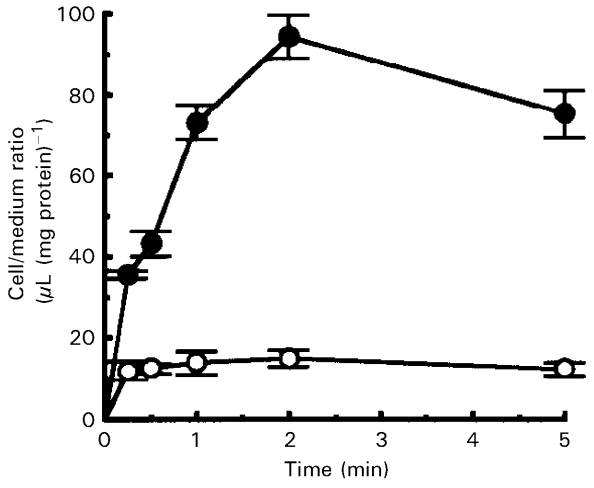

Figure 3. Time course of [^{14}C]benzoic acid uptake by rat MCT1 expressed in MDA-MB231 cells. Uptake of [^{14}C]benzoic acid by MDA-MB231 cells transfected with rat MCT1 (●) or with pRc-CMV vector alone (○) was measured at pH 6·0 and 37°C. Each point represents the mean ± s.e.m. of results from four experiments.

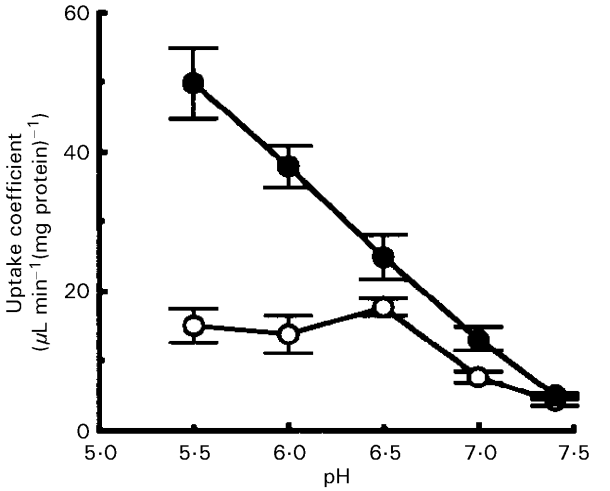

Figure 5. pH-Dependence of uptake of [^{14}C]benzoic acid (5·3 μ M) by rat MCT1 expressed in MDA-MB231 cells. Uptake of [^{14}C]benzoic acid was measured for MDA-17B231 cells transfected with rat MCT1 (●) or with pRc-CMV vector alone (○). Each point represents the means ± s.e.m. of results from four experiments.

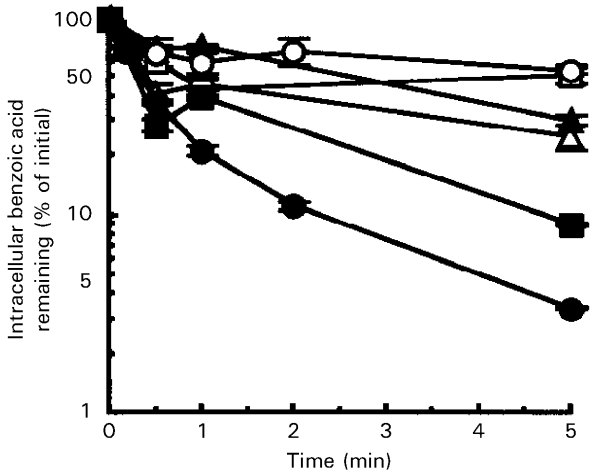

Figure 6. Time course of efflux of [^{14}C]benzoic acid by rat MCT1 expressed in MDA-MB231 cells. Cells were preloaded with [^{14}C]benzoic acid at 10 μM for 2 min, then efflux was initiated by incubating the cells in the buffer at pH 6·0 (▲, △), 7·0 (■, □) or 8·0 (●, ○). The results are shown as fractional amount remaining in the cells relative to the amount initially loaded in rat MCT1-transfected cells (▲, ■, ●) or pRc-CMV vector-transfected MDA-MB231 cells (△, □, ○). Each point represents the means ± s.e.m. of results from four experiments.

6 as the time-course of the fractional amount remaining in the cells. The efflux was enhanced when the efflux medium was at alkaline pH and was significantly higher in rat MCT1-transfected cells than in mock cells. Accordingly, rat MCT1 seems to transport benzoic acid bidirectionally.

Uptake of different compounds by rat MCT1-transfected cells

Table 1 shows the uptake of a variety of compounds by rat MCT1 and mock cells. In addition to L- and D-lactic acids and benzoic acid, uptake of acetic acid, propionic acid, butyric acid, nicotinic acid and salicylic acid was significantly higher in rat MCT1-transfected cells compared with mock cells. Phosphonoformic acid (foscarnet), with both carboxyl and phosphate groups within the molecule, was also transported by rat MCT1, whereas phosphoric acid was not, suggesting that the carboxyl group is important for recognition by rat MCT1. Increased uptake by rat MCT1 transfected cells was not observed for other monocarboxylic acids (such as mevalonic acid and faropenem), and other anionic compounds (glutamic acid, taurocholic acid, methotrexate) or a dipeptide (glycylsarcosine).

Discussion

We have previously postulated that some weak organic acids are transported, at least in part, by pH-dependent transporters in parallel with passive diffusion (Simanjuntak et al 1990, 1991; Tsuji et al 1990, 1994; Takanaga et al 1994, 1996; Tamai et al 1995a, 1997). In this study we examined, by use of cells transfected with MCT1 cloned from rat intestine, whether or not a typical weak organic

Table 1. Uptake of different compounds by cells transfected with rat MCT1 or pRc-CMV vector alone.

Compound	Concn (μM)	Uptake coefficient (μL (mg protein)$^{-1}$)	
		Rat MCT1	pRc-CMV
[^{14}C]Acetic acid	6	58·3 ± 2·26*	47·1 ± 3·45
[^{14}C]*p*-Aminohippuric acid	9	1·33 ± 0·11	1·28 ± 0·17
[^{14}C]Benzoic acid	60	75·6 ± 3·69*	7·13 ± 0·45
[^{14}C]Butyric acid	137	45·6 ± 5·32*	22·1 ± 2·37
[^{14}C]Faropenem	76	0·49 ± 0·12	0·42 ± 0·08
[^{14}C]Foscarnet	3	7·69 ± 0·79*	3·18 ± 0·61
[^{3}H]Glutamic acid	0·01	12·1 ± 0·36	16·8 ± 0·31
[^{14}C]Glycylsarcosine	17	2·50 ± 0·25	3·70 ± 0·53
L-[^{14}C]Lactic acid	3	40·2 ± 2·35*	3·53 ± 0·37
D-[^{14}C]Lactic acid	3	13·3 ± 1·56*	1·81 ± 0·07
[^{3}H]Methotrexate	0·03	10·8 ± 0·13	9·70 ± 1·28
[^{14}C]Mevalonic acid	67	1·63 ± 0·33	2·62 ± 0·15
[^{14}C]Nicotinic acid	7	36·8 ± 0·60*	29·2 ± 1·82
[^{14}C]Propionic acid	7	97·2 ± 6·93*	61·5 ± 2·57
[^{14}C]Salicylic acid	9	40·3 ± 1·83*	28·7 ± 1·63
[^{3}H]Taurocholic acid	0·65	1·54 ± 0·13	1·36 ± 0·20
[^{3}H]Valproic acid	19	3·47 ± 0·64	2·30 ± 0·79
H_3[^{32}P]O_4	5	17·7 ± 0·29	18·0 ± 1·23

Uptake of each compound by MDA-MB231 cells transfected with rat MCT1 or with pRc-CMV vector alone was measured for 2 min at pH 6·0 and 37°C at the concentration shown. Uptake of lactic acid was measured at 4°C. Data are means ± s.e.m. from four to six determinations. *$P < 0·05$ compared with cells transfected with pRc-CMV vector alone (*t*-test).

acid, benzoic acid, which has been used as a model compound for studies of passive diffusion, is a substrate of the pH-dependent monocarboxylic acid transporter MCT1. Furthermore, to confirm the potential pharmacological relevance of the transporter to the intestinal absorption of weak organic acid drugs, the intestinal tissue distribution of MCT1 has been clarified by immunohistochemical studies.

Firstly, we confirmed by immunohistochemical analysis that MCT1 protein is present in small intestinal tissues (Figure 1). Among intestinal tissues, MCT1 protein tended to be located on basolateral membranes in relatively immature epithelial cells such as parietal and cryptic cells whereas in mature absorptive epithelial cells its location shifted to the apical membrane. It was also confirmed (by Western blot analysis, data not shown) that MCT1 protein is present in the brush-border membrane fraction. This tissue distribution suggests MCT1 protein might perform different roles in different tissues. The intestinal flora produce large amounts of fermentation metabolites, including acetic, propionic and lactic acids, especially in the lower intestine. MCT1 might play a role in the absorption of such short-chain fatty acids. The expression of MCT1 beneath the tight junction might enable efficient transport of monocarboxylic acids from the intestinal lumen to the bloodstream. Because MCT transporters are generally known to transport monocarboxylic acids together with a proton, the direction of net transport is strongly dependent on the pH gradient across the cell membrane. Intestinal luminal acidic pH is advantageous as a driving force for proton-coupled transport of various monocarboxylic acids.

Secondly, we established a cell line that stably expresses rat MCT1 in MDA-MB231 cells. The membrane transport characteristics of lactic acid, a typical substrate of MCT1, were consistent with those found in our previous studies and by others using MCT1 cloned from rat and other animal species (Carpenter & Halestrap 1994; Takanaga et al 1995; Bröer et al 1997). They include saturability with a K_m value of 1–5 mM (approx.), stereospecificity with higher activity for the L-isomer and pH-dependence with greater activity at acidic pH (Figures 2A–C). These observations demonstrate that MCT1 expressed in the cell line established in this study retains the fundamental characteristics of MCT1 and is suitable for functional studies of rat MCT1.

Benzoic acid is an weak organic acid, pKa 4·19, and is a typical compound for which membrane transport is explained in terms of passive diffusion according to the pH–partition hypothesis. However, this study clearly demonstrated that benzoic acid is a substrate of the pH-dependent transporter MCT1. In rat MCT1-expressing cells uptake of [^{14}C]benzoic acid was significantly increased ca tenfold. The K_m value of benzoic acid transport via rat MCT1 is 3·05 mM, comparable with our previous observation in adenocarcinoma-derived Caco-2 cells from man, 4·83 mM at pH 6·0 (Tsuji et al 1994). Furthermore, the pH profile of initial uptake by rat MCT1 was clearly indicative of pH-dependence, whereas the uptake of [^{14}C]benzoic acid by mock (vector-transfected) cells was not significantly enhanced at acidic pH. These observations strongly support the idea that benzoic acid is transported by MCT1. Several other weak organic acids, e.g. short-chain fatty acids, nicotinic acid and salicylic acid were also transported by MCT1, whereas mevalonic acid and other acidic compounds were not recognized by this transporter. Accordingly, we have succeeded in demonstrating that in intestinal absorption MCT1 might be involved, at least in part, in the pH-dependent transport of certain (though not all) monocarboxylic acids which have previously been considered to be absorbed by passive diffusion. Takagi et al (1998) recently showed that transport of salicylic acid across the lipid bilayer of artificial liposomes satisfies several criteria of pH-dependent carrier-mediated transport, including overshoot uptake, saturation kinetics and inhibitory effects of analogues. Their results suggest that a pH gradient imposed across a lipid membrane can produce apparent carrier-mediated-like transport features in the absence of a transporter protein. They suggested that such transport phenomena are possible for highly membrane-permeable compounds. As shown in Table 1, transport activity in mock cells and in rat MCT1-expressing cells varied among the substrates tested. In mock cells transport activity for salicylic acid was significantly higher than for benzoic acid. This observation might reflect the involvement of such non-mediated but pH-dependent transport. In contrast, the significant increase in benzoic acid transport in cells expressing rat MCT1 suggests that pH-dependent carrier-mediated transport is more important in pH-dependent transport than is the putative non-carrier-mediated transport, although a diffusive non-carrier-mediated transport mechanism must be involved in part in the apparent total transport.

MCT1-mediated transport was bidirectional, as shown in the efflux study with [^{14}C]benzoic acid (Figure 6). MCT1 is present at the basolateral and brush-border membranes (Figure 1). Accordingly, MCT1 might control the exchange of native monocarboxylic acids such as lactic acid and

pyruvic acid between the bloodstream and intraepithelial cells and between intraepithelial cells and the intestinal lumen, as required. Garcia et al (1994) suggested that high levels of MCT1 on the basolateral membrane of epithelial cells might mediate net lactate export, because the intestine is believed to convert large amounts of glucose to lactate after a carbohydrate-rich meal. Alternatively, MCT1 might participate in the uptake of energy sources from the bloodstream by immature cells. Further studies are needed to clarify the physiological roles of MCT1 in the intestine.

In conclusion, MCT1 is present in epithelial cell membranes throughout the intestine and transports not only physiological weak acids, but also exogenous compounds such as benzoic acid. Accordingly, pH-dependent transport of some monocarboxylic acids both in intestinal brush-border and in basolateral membranes can be ascribed at least in part to the MCT1 protein. However, complete understanding of the pH-dependent transport mechanisms involved in intestinal epithelial cells will require further studies, in particular to establish the relative contributions of transporters and non-carrier-mediated transport in-vivo.

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