H⁺-peptide cotransport in the human bile duct epithelium cell line SK-ChA-1

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1Institute of Biochemistry, Department of Biochemistry/Biotechnology and 2Biozentrum of the Martin Luther University Halle-Wittenberg, Halle D-06120; and 3Molecular Nutrition Unit, Institute of Nutritional Sciences, Technical University of Munich, Freising-Weihenstephan, D-85350 Germany

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Knüter, Ilka, Isabel Rubio-Aliaga, Michael Boll, Gerd Hause, Hannelore Daniel, Klaus Neubert, and Matthias Brandsch. H⁺-peptide cotransport in the human bile duct epithelium cell line SK-ChA-1. Am J Physiol Gastrointest Liver Physiol 283: G222–G229, 2002.—This study describes for the first time the presence of H⁺-peptide cotransport in cells of the bile duct. Uptake of [glycine-1-14C]glycylsarcosine ([14C]Gly-Sar) in human extrahepatic cholangiocarcinoma SK-ChA-1 cells was stimulated sevenfold by an inwardly directed H⁺ gradient. Transport was mediated by a low-affinity system with a transport constant (Kᵣ) value of 1.1 mM. Several dipeptides, cefadroxil, and δ-aminolevulinic acid, but not glycine and glutathione, were strong inhibitors of Gly-Sar uptake. SK-ChA-1 cells formed tight, polarized monolayers on permeable membranes. The transepithelial electrical resistance was 856 ± 29 Ω cm². The transepithelial flux of [14C]Gly-Sar in apical-to-basolateral direction exceeded the basolateral-to-apical flux 11-fold. Uptake was 20-fold higher from the apical side. RT-PCR analysis using primer pairs specific for the intestinal-type peptide transporter (PEPT1) or kidney-type (PEPT2) revealed that the transport system expressed in SK-ChA-1 and also in cells of the native rabbit bile duct is PEPT1. Immunohistochemistry localized PEPT1 to the apical membrane of cholangiocytes of mouse extrahepatic biliary duct. We conclude that the cells of the mammalian extrahepatic biliary tract epithelium express the intestinal-type H⁺-peptide cotransporter in their apical membrane. SK-ChA-1 cells represent a convenient model to study the physiological and clinical aspects of peptide transport in cholangiocytes.

IN THE MAMMALIAN INTESTINE and kidney, transport of di- and tripeptides across the luminal membrane of epithelial cells occurs via carrier-mediated mechanisms energized by an inwardly directed H⁺ gradient (1, 12, 14, 16). It has been shown that the absorptive cells of the intestinal epithelium express the low-affinity system PEPT1, whereas the renal epithelium predominantly expresses the high-affinity system PEPT2 but also PEPT1. At the intestinal epithelium, PEPT1 is responsible for the absorption of di- and tripeptides originating from external dietary protein digestion. At the renal proximal tubulus, PEPT1 and PEPT2 are responsible for the reuptake of filtered peptides. In addition to their natural substrates, both systems are capable of transporting structurally related pharmacologically active compounds such as β-lactam antibiotics and other peptidomimetics (4, 27). Several cell lines such as Caco-2 (11), MDCK (9), SKPT (8), and LLC-PK1 (27) have been proven to be very useful tools for the investigation of function, mechanism, specificity, and regulatory aspects of peptide transport. In addition to intestine and kidney, specific mRNAs for H⁺-dependent peptide transporters have been found in brain, lung, pancreas, and liver (14, 16). To our knowledge, nothing has been published so far about peptide transport in cholangiocytes, the epithelial cells of the bile duct. This epithelium, however, has gained much attention in recent years. There have been major advances in our understanding of physiology and pathophysiology of this barrier (2, 22). Several transport systems have been described, e.g., Cl⁻/HCO₃⁻ exchangers, Na⁺/H⁺ exchangers, the CAMP-dependent Cl⁻ channel CFTR, aquaporin-1, a Na⁺-dependent glucose transporter and the Na⁺-dependent bile acid transporter (2). The transporters of the biliary epithelium are regulated by hormones and neuropeptides (2, 3).

In 1985, three cholangiocarcinoma cell lines were established and characterized in permanent tissue culture (18). Recently, they have been used for studies of Cl⁻ and K⁺ transport (7). In the present study, we characterize the uptake of [glycine-1-14C]glycylsarcosine ([14C]Gly-Sar) in SK-ChA-1 cells. Results reveal that SK-ChA-1 cells express the H⁺-dependent low-affinity transport system for di- and tripeptides PEPT1. The system is also expressed in normal rabbit and mouse extrahepatic bile duct cells. This study represents the first description of a H⁺/peptide transport in cells of the biliary duct.

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MATERIALS AND METHODS

Cell culture. The human extrahepatic biliary duct tumor cell line SK-ChA-1 established by Knuth et al. (18) was obtained from the Ludwig Institute for Cancer Research (Zurich, Switzerland). The human colon carcinoma cell line Caco-2 was obtained from the German Collection of Microorganisms and Cell Cultures (Braunschweig, Germany). Cells at passage 27-65 (SK-ChA-1) or 18-23 (Caco-2) were maintained in 75-cm² culture flasks at 37°C in a humidified atmosphere with 5% CO₂. They were cultured in minimum essential medium supplemented with nonessential amino acid solution (1%), fetal bovine serum (10%), and gentamicin (50 μg/ml). All cell culture media were purchased from Life Technologies (Karlsruhe, Germany). Cells grown to confluence were released by trypsinization (0.05% trypsin/EDTA in 50 °C). The human colon carcinoma cell line SK-ChA-1 cells were also cultured on permeable polycarbonate Transwell cell culture inserts (24.5-mm diameter, 3 μm pore size; Costar, Bodenheim, Germany). Subcultures were started at a cell density of 43,000 cells/cm² and cultured for 14–18 days. The lower (receiver) compartment contained 2.6 ml medium and the upper (donor) compartment, 1.5 ml medium.

Transport studies. Uptake of [14C]Gly-Sar (53 mCi/mmol specific radioactivity; Amersham International) was determined at 37°C. In most experiments, the uptake medium was 25 mM 2-(N-morpholino)-ethanesulfonic acid/Tris, pH 6.0, or 25 mM HEPES/Tris, pH 7.5, containing (in mM): 140 NaCl, 5.4 KCl, 1.8 CaCl₂, 0.8 MgSO₄, and 5 glucose. Na⁺-free media were prepared by replacing NaCl in the uptake medium by chloring chloride. The procedure for NH₄Cl prepulse was the same as described previously (8, 9, 11, 21). Uptake was initiated by removing the pretreatment culture medium from the dish, washing the cell layer with 1 ml buffer and adding 1-ml uptake medium containing [14C]Gly-Sar (8–11, 15). After incubation for the desired time in the presence or absence of unlabeled compounds (Sigma-Aldrich), the buffer was removed, and monolayers were quickly washed four times with ice-cold uptake buffer, dissolved, and transferred to counting vials. Radioactivity associated with the cells was measured by liquid scintillation spectrometry. Transepithelial flux of [14C]Gly-Sar across SK-ChA-1 cell monolayers cultured on permeable filters was measured as follows. After washing the inserts with buffer (in mM): 25 mM HEPES/Tris (pH 7.5), 140 NaCl, 5.4 KCl, 1.8 CaCl₂, 0.8 MgSO₄, and 5 glucose for 10 min, uptake was started by adding uptake buffer (pH 6.0) containing [14C]Gly-Sar to the donor side (1.5 ml apical or 2.6 ml basolateral compartment, respectively). All experiments were performed at 37°C in a shaking water bath. At time intervals of 10, 30, 60, and 120 min, samples were taken from the receiver compartment and replaced with fresh buffer. Radioactivity in the samples was measured by liquid scintillation spectrometry. After 2 h, the filters were quickly washed four times with ice-cold uptake buffer, cut out of the plastic insert and transferred to scintillation vials. The integrity of the SK-ChA-1 grown on permeable filters was confirmed microscopically, by measuring the transepithelial electrical resistance and by measuring the apical-to-basolateral transepithelial flux of [14C]mannitol (10 μM) as a space marker.

Transmission electron microscopy. SK-ChA-1 cells cultured on polycarbonate cell culture inserts for 14 days were fixed in 3% sodiumcacodylate-buffered glutaraldehyde (pH 7.2), postfixed with 1% OsO₄ solution, dehydrated in an ethanol series, and embedded in epoxy resin (23). Ultrathin sections (90 nm) were stained with uranyl acetate/lead citrate and observed with an EM 900 transmission electron microscope (Zeiss).

RNA isolation and RT-PCR. SK-ChA-1 cells were cultured in 75-cm² culture flasks for 7 days. Total RNA from the cell line and from rabbit tissues (intestinal mucosa, kidney, gall bladder, and bile duct; Charles River Laboratories) was isolated with the RNAwiz system according to the manufacturer's protocol (Ambion, Wiesbaden, Germany). Two micrograms total RNA were reverse transcribed using the Retroscript kit (Ambion). Five microliters of each RT reaction were subjected to PCR reactions (REDAq; Sigma) with the following primer pairs derived from mouse: 1) PEPT1-F2 (5'-GAGCATCCTCT-TCATCGTGTC-3') and PEPT1-B901 (5'-CGCTGCTGGTCAACAGGCGC-3'); 2) PEPT2-F290 (5'-ACCATGTCCTACGC-GCCTC-3') and PEPT2-B1161 (5'-CGTGGATCATACCAACAGC-3'); 3) GAPDH-F (5'-GACCAGACTGGATCAC-GCCT-3') and GAPDH-B (5'-TCCACACCCGTCTTGTATAGG-3') in 25 μl total volume. PCR conditions were: 94°C 1 min, 35× (94°C 30 s, 57°C 30 s, 72°C 45 s). Ten microliters of each PCR reaction were separated on a 1% agarose gel.

Immunohistochemistry. Murine tissues were fixed in 4% paraformaldehyde overnight at 4°C and processed for embedding in paraffin wax. Deparaffinized sections (5 μm) were used for immunofluorescence analysis. Antigen retrieval was carried out by incubating the slides in citrate buffer (pH 6.0) in a microwave oven. Slides were blocked for 20 min with 3% goat serum and incubated overnight with a rabbit polyclonal anti-mouse-PEPT1 serum raised against the amino acids 248–261 (17) diluted 1:100. For detection of the primary antibody, the slides were incubated 1 h with an anti-rabbit Cy3 coupled antibody (1:200; Dianova). Control incubations in parallel sections were carried out to specify the reaction by preabsorption of the primary antibody with 5 μg of the corresponding antigenic peptide. Slides were viewed using confocal laser scanning microscopy (model TCS SP2; Leica Microsystems, Heidelberg, Germany).

Data analysis. Each experimental point shown represents the mean ± SE of three to four measurements. The kinetic constants were calculated by nonlinear regression of the Michaelis-Menten plot and confirmed by linear regression of the Eadie-Hofstee plot. Calculated parameters are shown with their SE. Inhibition constants (Ki) were calculated from IC50 values (i.e., concentration of the unlabeled compound necessary to inhibit 50% of radiolabeled Gly-Sar uptake).

RESULTS

Effect of a pH gradient on the [14C]Gly-Sar uptake in SK-ChA-1 cells. To determine whether epithelial cells of the mammalian bile duct express a H⁺/peptide symport system, we studied the uptake of [14C]Gly-Sar in the human cholangiocarcinoma cell line SK-ChA-1, cultured as monolayers on impermeable plastic surfaces. The uptake activity, expressed as pmol·10⁻⁶ g protein⁻¹, remained approximately the same for ≤12 days (data not shown). Day 7 was chosen for all uptake experiments. [14C]Gly-Sar uptake was markedly stimulated by changing the extracellular pH (Fig. 1). Uptake measured at pH 6.0 was sevenfold greater than uptake measured at pH 8.5. An extracellular pH of 6.0 represents the optimum for the Gly-Sar uptake. The same pH optimum for H⁺-dependent
H⁺-peptide cotransport has been found in the intestinal cell line Caco-2 and in the renal cell lines MDCK and SKPT (8–11). To determine whether the stimulation observed at pH 6.0 was due to the inwardly directed H⁺ gradient or due to the acidic pH per se, we investigated the influence of intracellular pH on [¹⁴C]Gly-Sar uptake. Intracellular pH was decreased by the NH₄Cl prepulse technique (21). Results of these experiments were performed on both SK-ChA-1 and, for comparison, Caco-2 cells are given in Table 1. [¹⁴C]Gly-Sar uptake at an extracellular pH of 6.0 is inhibited by 70% in both cell lines when the intracellular pH was made acidic. Stimulation of Gly-Sar uptake caused by an acidic extracellular pH is thus the effect of an inwardly directed H⁺ gradient rather than the acidic extracellular pH per se. This conclusion is further supported by the finding that the protonophore carbonyl cyanide p-(trifluoromethoxy)phenylhydra-

Table 1. Influence of an inwardly directed H⁺ gradient on [¹⁴C]Gly-Sar uptake in SK-ChA-1 and Caco-2 cells

<table>
<thead>
<tr>
<th>Intracellular pH</th>
<th>[¹⁴C]Gly-Sar Uptake, pmol·10⁻⁶ min⁻¹·mg protein⁻¹</th>
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</thead>
<tbody>
<tr>
<td>Normal</td>
<td>SK-ChA-1: 200 ± 3 (100%) Caco-2: 289 ± 2 (100%)</td>
</tr>
<tr>
<td>Acidic</td>
<td>SK-ChA-1: 27.6 ± 1.4 (14%) Caco-2: 79.3 ± 2.8 (27%)</td>
</tr>
</tbody>
</table>

Data represent means ± SE for 4 determinations. Confluent monolayer cultures of SK-ChA-1 or Caco-2 cells were treated at 37°C for 15 min with either the control buffer (in mM); 25 HEPES/Tris, 5.4 KCl, 1.8 CaCl₂, 0.8 MgSO₄, 5 glucose, 140 choline chloride, pH 7.5 or the NH₄Cl-containing buffer (in mM); 25 HEPES/Tris, 5.4 KCl, 1.8 CaCl₂, 0.8 MgSO₄, 5 glucose, 115 choline chloride, 25 NH₄Cl pH 7.5. After treatment, the monolayers were washed 3 times with the NH₄Cl-free uptake buffer and uptake of [glycine-¹⁴C]glycylsarcosine ([¹⁴C]Gly-Sar) was measured. Uptake medium used for [¹⁴C]Gly-Sar was (in mM); 140 choline chloride, 5.4 KCl, 1.8 CaCl₂, 0.8 MgSO₄, 5 glucose buffered with 25 MES/Tris, pH 6.0. Concentration of [¹⁴C]Gly-Sar was 10 μM.

Fig. 1. pH dependence of [glycine-¹⁴C]glycylsarcosine ([¹⁴C]Gly-Sar) uptake in SK-ChA-1 cells. Uptake of [¹⁴C]Gly-Sar (10 μM) was measured at varying pH values (range 5.0–9.0). Values represent means ± SE for 3 determinations. When not indicated, the error lies within the symbol.

Fig. 2. Kinetics of Gly-Sar uptake in SK-ChA-1 cells. Uptake of [¹⁴C]Gly-Sar was measured with a 10-min incubation over a Gly-Sar concentration range of 20 μM–10 mM. The diffusional/binding component was determined by measuring uptake in the presence of an excess amount (50 mM) of unlabeled Gly-Sar. This component was 10.9% of total uptake at 20 μM of [¹⁴C]Gly-Sar. The relationship between carrier-mediated uptake rate and substrate concentration was found to be hyperbolic over the Gly-Sar concentration range (Fig. 2), indicating saturability of the transport system. When the results were expressed in the form of an Eadie-Hofstee plot (uptake rate/substrate concentration vs. uptake rate), a straight line (r² = 0.98) was obtained (Fig. 2, inset). The apparent Michaelis-Menten constant of transport processes (Km) was 1.1 ± 0.1 mM and the maximal velocity (Vmax) was 21.6 ± 2.2 nmol·10⁻⁶ min⁻¹·mg protein⁻¹. We found no evidence for the presence of a high affinity/low capacity transport system as described in SKPT cells (8) and in renal brush-border membrane vesicles (12).

Substrate specificity. To determine the substrate specificity of the H⁺-dependent transport system re-

zone (FCCP, 10 μM, present during uptake measurement), which dissipates transmembrane electrochemical H⁺ gradients, inhibited [¹⁴C]Gly-Sar uptake from 199 ± 8 to 95.2 ± 8.2 pmol·10⁻⁶·mg protein⁻¹ (by 52%) in SK-ChA-1 cells and from 237 ± 9 to 95.5 ± 18.7 pmol·10⁻⁶·mg protein⁻¹ (by 60%) in Caco-2 cells.

Kinetic parameters. Dependence of the Gly-Sar uptake rate on the substrate concentration was investigated to determine the kinetic parameters of the transport system. Uptake rates were measured over a substrate concentration range of 20 μM–10 mM. Carrier-mediated uptake calculated by subtracting the nonmediated component from the total uptake was used in the kinetic analysis. The nonmediated component, which represents diffusion plus binding, was determined from the uptake of [¹⁴C]Gly-Sar in the presence of excess amount (50 mM) of unlabeled Gly-Sar. This component was 10.9% of total uptake at 20 μM of [¹⁴C]Gly-Sar. The relationship between carrier-mediated uptake rate and substrate concentration was found to be hyperbolic over the Gly-Sar concentration range (Fig. 2), indicating saturability of the transport system.

Data represent means ± SE for 4 determinations. Inset: Eadie-Hofstee transformation of the data. V, Uptake rate in nmol·10⁻⁶·mg protein⁻¹; S, Gly-Sar concentration in mM.
sponsible for the uptake of Gly-Sar in SK-ChA-1 cells, the effect of unlabeled peptides, peptidomimetics, and glycine on the uptake of Gly-Sar was measured at pH 6.0 (Fig. 3). Table 2 shows the resulting $K_i$ values. $K_i$ values of potential substrates range from 0.25 ± 0.01 mM for Ala-Ala to 3.3 ± 0.3 mM for δ-aminolevulinic acid. Glycine and glutathione were not recognized. Results indicate that dipeptides and several peptidomimetics are recognized by the peptide transport system as potential transport substrates.

**Transepithelial flux.** The inner layer of the extrahepatic bile duct is formed by cholangiocytes in polarized, epithelial formation. Microscopic studies by Knuth et al. (18) have shown that SK-ChA-1 cells also polarize when grown on cover glasses. Here, we cultured SK-ChA-1 cells on permeable filters for transepithelial flux studies. Transmission electron micrographs (Fig. 4) show that within 14 days the cells form an epithelial monolayer with polarized cells. They establish a brush border at their apical membrane and form junctional complexes between cells. Transport studies were performed after 18 days. At this stage, the transepithelial electrical resistance of the SK-ChA-1 monolayers was 856 ± 29 Ω × cm². The transepithelial flux of $[^{14}C]$mannitol through the SK-ChA-1 cell monolayers was 0.37 ± 0.01%·h⁻¹·receiver well⁻¹. These results demonstrate that SK-ChA-1 cell monolayers on permeable filters are well suited as a model for biliary tract flux studies as are Caco-2 cells for studies of intestinal transport. Figure 5 summarizes the results of Gly-Sar flux studies. When added to the basolateral compartment in an uptake buffer (pH 6.0), the $[^{14}C]$Gly-Sar flux to the apical compartment is only insignificantly higher than the flux of the space marker $[^{14}C]$mannitol. However, from the apical side, transepithelial $[^{14}C]$Gly-Sar flux to the basolateral side (5.6 ± 0.4%·h⁻¹·receiver well⁻¹) exceeds the $[^{14}C]$mannitol flux 15-fold and the basolateral-to-apical $[^{14}C]$Gly-Sar flux 11-fold. Hence, the $[^{14}C]$Gly-Sar transport is dominantly directed absorptively, and almost neglectable in the excretory direction. As expected, the transepithelial flux of $[^{14}C]$Gly-Sar is mainly carrier-mediated. Addition of an excess amount of unlabeled Gly-Sar (10 mM) to the apical compartment inhibits the apical-to-basolateral $[^{14}C]$Gly-Sar flux by 79% (from 5.6 ± 0.4 to 1.2 ± 0.07%·h⁻¹·receiver well⁻¹). The flux results correspond very well with the uptake into the cells on the filter. These filters were cut out after 2 h and analyzed. Figure 5 (inset) shows that the $[^{14}C]$Gly-Sar uptake from the apical side exceeds the uptake from the basolateral side by a factor of 20. Unlabeled Gly-Sar at a concentration of 10 mM at the apical side inhibits the apical $[^{14}C]$Gly-Sar uptake into the cells by 78%.

**Expression of PEPT1 in SK-ChA-1 cells and native extrahepatic biliary duct.** Distinct H⁺-peptide cotransporters have been cloned from human tissues: PEPT1 from intestine and PEPT2 from the kidney epithelial cells (1, 4, 12, 16). To identify conclusively the peptide transporter found in SK-ChA-1 cells, RT-PCR analysis of mRNA isolated from these cells using primers specific for PEPT1 and PEPT2, respectively, was carried out. Results are given in Fig. 6. As expected, PEPT1 is

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**Table 2. Inhibition constants ($K_i$) for different peptides and peptidomimetics for the inhibition of $[^{14}C]$Gly-Sar uptake in SK-ChA-1 cells**

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>$K_i$ (mM)</th>
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<tbody>
<tr>
<td>Glycine</td>
<td>&gt;30</td>
</tr>
<tr>
<td>Gly-Sar</td>
<td>0.78 ± 0.1</td>
</tr>
<tr>
<td>Ala-Ala</td>
<td>0.25 ± 0.01</td>
</tr>
<tr>
<td>Ala-Asp</td>
<td>0.37 ± 0.02</td>
</tr>
<tr>
<td>Cefadroxil</td>
<td>3.1 ± 0.2</td>
</tr>
<tr>
<td>δ-Aminolevulinic acid</td>
<td>3.3 ± 0.3</td>
</tr>
<tr>
<td>Glutathione</td>
<td>&gt;30</td>
</tr>
</tbody>
</table>

Values are in mM, means ± SE. Uptake of $[^{14}C]$Gly-Sar (10 μM) was measured in confluent cultures of SK-ChA-1 cells for 10 min at pH 6.0. $K_i$ values ± SE were calculated from IC₅₀ values derived by nonlinear regression analysis of data shown in Fig. 3.
expressed both in the small intestine and the kidney. PEPT2 is expressed only in the kidney but not in the intestine. SK-ChA-1 cells, cells of the gall bladder epithelium, and cells of native rabbit extrahepatic biliary duct express PEPT1.

Transwell experiments gave strong functional evidence for the expression of PEPT1 at the apical side of cholangiocytes. This is supported by immunohistochemistry using a polyclonal antibody specific for PEPT1 (17) (Fig. 7). PEPT1 is localized to cholangio-
cytes of mouse extrahepatic biliary duct with the highest intensity at the apical membrane.

DISCUSSION

So far, in mammalian tissues, H⁺/peptide cotransport activity has been found by functional assays in kidney and intestine and mostly on the mRNA level in brain, liver, lung, and pancreas. In our present study, we describe for the first time, a H⁺/dependent peptide cotransport system in the extrahepatic biliary duct. SK-ChA-1 cells express a system in their apical membrane, which transports Gly-Sar in a pH-dependent manner into the cell. The existence of H⁺-Gly-Sar cotransport is evident from the results: 1) Gly-Sar transport is stimulated by an extracellular acidic pH; 2) inner acidification reduces Gly-Sar transport; and 3)
protonophore FCCP, which dissipates transmembrane electrochemical H+ gradients inhibits [14C]Gly-Sar uptake. The \( K_i \) value of Gly-Sar transport of 1.1 mM qualifies the system responsible for Gly-Sar uptake in these cells as the low-affinity intestinal-type system PEPT1. \( K_i \) values of competing peptides and peptidomimetics also support the conclusion that the system expressed is PEPT1. RT-PCR analysis using RNA from several tissues confirmed the expression of PEPT1 in SK-ChA-1 cells and demonstrated that the expression of the H+/peptide symporter PEPT1 is a physiologically occurring fact in rabbit bile duct tissue not restricted to the tumorous biliary epithelial cell line. From analysis of the kinetic studies and the PCR results, we conclude that at extrahepatic bile duct cells, predominantly PEPT1 is expressed. We cannot rule out, however, that in addition to PEPT1, PEPT2 is coexpressed to a very minor extent. From transepithelial flux studies showing that flux and uptake of [14C]Gly-Sar is carrier-mediated and that apical-to-basolateral flux and apical uptake exceeded the flux in the opposite direction and the basolateral uptake 11- to 20-fold, we postulate that the carrier is located in the apical membrane of SK-ChA-1 cells. This was confirmed by immunohistochemistry. By using a PEPT1 antibody, we localized PEPT1 to the apical membrane of mouse bile duct epithelial cells.

What could be the physiological function of peptide transport at the biliary epithelium? Very little is known about the presence of small peptides in bile fluid. Glutathione is secreted into bile and is almost completely broken down (6). The authors of this study suggested that the resulting products are reabsorbed either as peptides, conjugates, or free amino acids. Furthermore, efficient hepatobiliary excretion has been described for amino-acylated di- and tripeptides (5). Glutathione and N-protected di- and tripeptides, however, do not represent substrates for H+/peptide cotransporters. Lacking knowledge about the presence of di- and tripeptides in bile fluid does not necessarily mean that the concentration of potential substrates for peptide transporters in the biliary epithelium is negligible. For example, by the use of reverse-phase chromatography, mass spectrometry and Edman degradation, several hydrophobic polypeptides have been unexpectedly identified in human bile (25). Furthermore, biologically active peptides, such as atrial natriuretic factor, have been found in bile fluid (19). A similar situation prevailed for many years regarding the physiological function of the H+/peptide cotransport (peptide reabsorption) process in the kidney. Concentration of small peptides in the circulation was considered to be very low until Seal and Parker (23) could show that the plasma levels of peptide-bound amino acids are manyfold higher than once thought. Therefore, it became obvious that the renal reabsorptive process for small peptides does play a significant role in the conservation of peptide-bound amino nitrogen under physiological conditions. It remains to be elucidated whether PEPT1 functions as a recovery system of di- and tripeptides excreted from hepatocytes into the bile. Further studies will be needed to clarify the existence of significant amounts of small peptides in bile. The potential pharmacological relevance of peptide transport is apparent from the observation that several pharmacologically active peptidomimetic drugs, such as certain \( \beta \)-lactam antibiotics, are substrates for this process (1, 4, 12, 16, 26). Not surprisingly, PEPT1 in SK-ChA-1 cells recognizes cefadroxil (\( K_i = 3.3 \) mM) as a potential substrate in our study. Of special interest is the observation that \( \delta \)-aminolevulinic acid is able to inhibit Gly-Sar uptake (\( K_i = 3.3 \) mM). This compound, a precursor of porphyrin synthesis used as an endogenous photosensitizer for photodynamic therapy of various tumors (20) has been shown to be a good substrate of intestinal and renal peptide transporters (13). This explains its high oral bioavailability. Accumulation of \( \delta \)-aminolevulinic acid via PEPT1 in bile duct epithelial cells would allow the use of this compound for treatment of extrahepatic biliary tract carcinoma (28).

In conclusion, mammalian cholangiocytes express the H+/peptide symporter PEPT1 in their apical membranes. SK-ChA-1 cells represent a convenient model to study both the physiological role and the possible clinical applications of the peptide transport system in the extrahepatic biliary tract epithelium.

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