Functional expression of novel peptide transporter in renal basolateral membranes

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Terada, Tomohiro, Kyoko Sawada, Tatsuya Ito, Hideyuki Saito, Yukiya Hashimoto, and Ken-ichi Inui. Functional expression of novel peptide transporter in renal basolateral membranes. Am J Physiol Renal Physiol 279: F851–F857, 2000.—We examined the peptide transport activity in renal basolateral membranes. [14C]glycylsarcosine (Gly-Sar) uptake in rat renal cortical slices was saturable and inhibited by excess dipeptide and aminocephalosporin cefadroxil. When several renal cell lines were screened for the basolateral peptide transport activity, Madin-Darby canine kidney (MDCK) cells were demonstrated to have the greatest transport activity. [14C]Gly-Sar uptake across the basolateral membranes of MDCK cells was inhibited by diand tripeptide and decreased with decreases in extracellular pH from 7.4 to 5.0. The Michaelis-Menten constant value of [14C]Gly-Sar uptake across the basolateral membranes of MDCK cells was 71 µM. The basolateral peptide transporter in MDCK cells showed several different [14C]Gly-Sar transport characteristics in growth dependence, pH profile, substrate affinity, and sensitivities to chemical modifiers from those of the apical H\(^+\)-peptide cotransporter of MDCK cells. The findings of the present investigation indicated that the peptide transporter was expressed in the renal basolateral membranes. In addition, from the functional characteristics, the renal basolateral peptide transporter was suggested to be distinguishable from known peptide transporters, i.e., H\(^+\)-peptide cotransporters (PEPT1 and PEPT2) and the intestinal basolateral peptide transporter.

The kidney plays an important role in the metabolism of circulating small peptides (1, 8). In 1977, Adibi et al. (2) reported that the kidney possessed the greatest capacity to transport and metabolize intravenously injected dipeptide among the tissues examined (kidney, intestine, liver, and skeletal muscles). Subsequently, several studies using isolated perfused kidney have demonstrated that the renal metabolism of dipeptides mainly occurs on the luminal side, i.e., glomerular filtration, intraluminal hydrolysis, and luminal uptake into the cells (7, 19, 21). However, it was also suggested that peritubular metabolism, in addition to luminal metabolism, contributed to the renal clearance of dipeptides, because the amount of peptide filtrated was less than the amount of peptides that disappeared from the perfusate (7, 19). Although the mechanisms involved in this peritubular process were not clarified at that time, the cellular uptake through the basolateral membranes was suggested to be involved in this metabolism. Furthermore, Nutzenadel and Scriver (22) reported the uptake of L-carnosine by rat renal cortical slices. Because renal cortical slices predominantly expose the basolateral membranes to the medium (3), the authors suggested that the uptake characteristics reflected the transport processes through the basolateral side rather than through the luminal side (22).

Thereafter, with respect to the investigation on the renal handling of small peptides, numerous efforts have been devoted to characterize the luminal transport systems using a variety of experimental techniques. The molecular nature of these transport systems has been clarified by the identification of two kinds of H\(^+\)-peptide cotransporters, PEPT1 and PEPT2 (1, 6, 15, 18). Immunohistochemical studies revealed that PEPT1 and PEPT2 were localized at the brush-border membranes of S1 and S3 segments of proximal tubules, respectively (25). In contrast to the luminal peptide transport systems, there is little functional and molecular information available on the peptide transport system localized at the basolateral membranes in the kidney. Therefore, to gain information about the renal basolateral peptide transporter, we examined the transport characteristics of [14C]glycylsarcosine (Gly-Sar) in rat renal cortical slices. In addition, to search cell lines expressing the renal basolateral peptide transporter, three kinds of widely used renal cell lines were screened for [14C]Gly-Sar uptake across the basolateral membranes. As a result, Madin-Darby canine kidney (MDCK) cells were demonstrated to show the greatest transport activity. Using MDCK cells, we further characterized the functional properties of the basolateral peptide transporter by comparing them with those of the apical peptide transporter, because MDCK cells are known to express the H\(^+\)-peptide cotransporter at the apical membranes (5, 29).
MATERIALS AND METHODS

Uptake in rat renal cortical slices. Preparation of rat renal cortical slices and uptake procedures were as described previously (13, 17, 31). Briefly, slices weighing 50–80 mg were randomly selected and placed in oxygenized incubation buffer [(in mM) 120 NaCl, 16.2 KCl, 1 CaCl₂, 1.2 MgSO₄ and 10 NaH₂PO₄/Na₂HPO₄, pH 7.5] containing [¹⁴C]Gly-Sar (1 or 5 μM) and D-[³H]mannitol (1 or 2.5 μM). D-[³H]mannitol was used to estimate the extracellular trapping and nonspecific uptake of [¹⁴C]Gly-Sar. After the incubation period, each slice was rapidly washed twice with ice-cold incubation buffer, blotted on filter paper and solubilized in 0.5 ml of NCS II (Amersham, Buckinghamshire, UK). Then, the radioactivity was determined in 10 ml of ACS II (Amersham) by liquid scintillation counting.

Cell culture. MDCK cells (32), LLC-PK₁ cells (24), and opossum kidney (OK) cells (14) were cultured, as previously described. To measure the uptake of [¹⁴C]Gly-Sar from the apical side of MDCK cells, 12-well microplates or 35-mm plastic dishes were inoculated with 1 × 10⁵ cells in 1 ml or with 2 × 10⁵ cells in 2 ml of culture medium, respectively. To measure the uptake of [¹⁴C]Gly-Sar from the basolateral side, OK, LLC-PK₁, and MDCK cells were seeded on micro-porous membrane filters (3-μm pores, 1 cm²) inside Transwell cell culture chambers (Costar, Cambridge, MA) at a cell density of 4 × 10⁵ cells/filter for all cells. The cell monolayers were given culture medium every 2–3 days and were used on the 6th day for uptake studies.

Uptake studies by monolayers. [¹⁴C]Gly-Sar uptake by monolayers grown in 12-well microplates and 35-mm plastic dishes was determined as described previously (27). [¹⁴C]Gly-Sar uptake by monolayers grown in the Transwell chambers was measured as described previously (28).

Statistical analysis. Each experimental point shown represents the mean ± SE of 3–12 measurements from 1–3 independent experiments. When the error bars are not shown, they are smaller than the symbol. Data were analyzed statistically by nonpaired t-test or one-way analysis of variance followed by Scheffe’s test when multiple comparisons were needed.

Materials. Cefadroxil was supplied from Bristol Meyers (Tokyo, Japan). [¹⁴C]Gly-Sar (1.75 GBq/mmol) was obtained from Daiichi Pure Chemicals (Ibaraki, Japan). D-[³H]mannitol (736.3 GBq/mmol) was obtained from NEN Life Science Products (Boston, MA). Diglycine, triglycine, tetruglycine, Gly-Sar, and p-chloromercuribenzenesulfonic acid (PCMBS) were obtained from Sigma (St. Louis, MO). Glycyl-L-leucine was purchased from Peptide Institute (Osaka, Japan). Diethylypyrocarbonate (DEPC) and glycine were obtained from Nacalai Tesque (Kyoto, Japan). All other chemicals used were of the highest purity available.

RESULTS

Gly-Sar uptake in rat renal cortical slices. Figure 1A shows the time course of [¹⁴C]Gly-Sar uptake in rat renal cortical slices. The [¹⁴C]Gly-Sar uptake was increased in a time-dependent manner and inhibited by excess unlabeled Gly-Sar at all times tested. The [¹⁴C]Gly-Sar uptake was not inhibited by glycine but was significantly inhibited by Gly-Sar and cefadroxil (Fig. 1B). The [¹⁴C]Gly-Sar uptake by rat renal cortical slices was saturable with an apparent Michaelis-Menten constant (Kₘ) value of 55 μM (Fig. 1C).

Screening of renal cell lines for Gly-Sar uptake across the basolateral membranes. We screened widely used renal cell lines for the basolateral peptide transport activity. Figure 2 shows the time course of [¹⁴C]Gly-Sar uptake across the basolateral membranes of OK (Fig. 2A), LLC-PK₁ (Fig. 2B), and MDCK cells (Fig. 2C). The specific uptake of Gly-Sar was the greatest in MDCK cells among the cell lines examined. [¹⁴C]Gly-Sar uptake from the apical side in MDCK cells was also
inhibited by 10 mM unlabeled Gly-Sar (Fig. 2D), indicating the expression of peptide transporter in the apical membranes of MDCK cells. In the following experiments, we performed a functional comparison of the apical and basolateral peptide transporters in MDCK cells.

Functional comparison of Gly-Sar uptake by the apical and basolateral peptide transporters in MDCK cells. Figure 3 shows the growth dependence of $[^{14}C]$Gly-Sar uptake by the apical and basolateral peptide transporters in MDCK cells. The $[^{14}C]$Gly-Sar uptake by both transporters was inhibited in the presence of 10 mM unlabeled Gly-Sar throughout the duration of the culture. The specific uptake of $[^{14}C]$Gly-Sar by the apical transporter gradually increased with the period of culture. In contrast, the specific uptake of $[^{14}C]$Gly-Sar by the basolateral peptide transporter was increased up to the 6th day and then decreased to the 10th day. All subsequent experiments for both transporters were done on the 6th day after seeding to gain the greatest transport activity of the basolateral peptide transporter.

To determine the substrate specificity, inhibition studies were carried out. The $[^{14}C]$Gly-Sar uptake by both transporters was markedly inhibited by 10 mM diglycine and triglycine, whereas glycine and tetruglycine at 10 mM did not show a significant inhibitory effect on $[^{14}C]$Gly-Sar uptake (Fig. 4).

Figure 5 shows the pH dependence of $[^{14}C]$Gly-Sar uptake by the apical and the basolateral peptide transporters in MDCK cells. At all pHs examined, $[^{14}C]$Gly-Sar uptake was suppressed in the presence of excess glycyl-L-leucine. As shown in insets of Fig. 5, the apical peptide transporter-mediated $[^{14}C]$Gly-Sar uptake was maximal at pH 6.0–6.5. In contrast, specific uptake of $[^{14}C]$Gly-Sar by the basolateral peptide transporter was decreased in accordance with decreases in pH from 7.4 to 5.0.

The $[^{14}C]$Gly-Sar uptakes by the apical and the basolateral peptide transporters in MDCK cells were both saturable. The apparent $K_m$ values for the apical and the basolateral peptide transporters were 440 and 71 $\mu$M, respectively (Fig. 6).

The histidine residue modifier, DEPC, and the sulfhydryl reagent, PCMBS, were reported to show different inhibitory effects on the apical and basolateral peptide transporters in the human intestinal cell line Caco-2 (16, 23, 28). As shown in Fig. 7, the magnitude of inhibition produced by DEPC was significantly greater on the apical transport rather than on the basolateral transport, and the basolateral peptide

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Fig. 2. Time course of $[^{14}C]$Gly-Sar uptake from basolateral side in opossum kidney (OK) (A), LLC-PK1 (B), and Madin-Darby canine kidney (MDCK) (C) cells, and the apical side in MDCK cells (D). Each cell was incubated with 20 $\mu$M $[^{14}C]$Gly-Sar added to either the basolateral (pH 7.4) or apical (pH 6.0) side in the absence (○) or presence (●) of 10 mM unlabeled Gly-Sar. Each point represents the mean ± SE of 3 monolayers.
DISCUSSION

Using human intestinal cell line Caco-2, we previously demonstrated that the intestinal basolateral peptide transporter, functionally distinguishable from the apical \( \text{H}^+ \)-peptide cotransporter (PEPT1), was involved in the transepithelial transport of small peptides and peptidyl drugs (16, 20, 23, 28). The intestinal basolateral peptide transporter was characterized as a low-affinity and facilitative transporter (23, 28). In contrast, although it was hypothesized that the renal basolateral peptide transporter was involved in the peritubular clearance of dipeptides (7, 19), the nature of this transporter has been little understood. Thus the purpose of the present study was to determine whether the basolateral peptide transporter functioned in the kidney.

Our present findings clearly demonstrated that the peptide transporter was functionally expressed in the basolateral membranes of rat kidney and MDCK cells. The pH profile of the renal basolateral peptide transporter is distinct from that of the intestinal basolateral peptide transporter. The basolateral peptide transporter in Caco-2 cells mediated \(^{14}\text{C}\)Gly-Sar uptake in an extracellular pH-independent manner (28). \( K_m \) values of Gly-Sar for the renal basolateral peptide transporters (55 \( \mu \text{M} \) in rat renal cortical slices and 71 \( \mu \text{M} \) in MDCK cells) were much smaller than that for the intestinal basolateral peptide transporter of Caco-2 cells (2.1 mM) (28). These results suggested that the renal and intestinal basolateral peptide transporters were different from each other. The low affinity of the intestinal basolateral peptide transporter was assumed to be advantageous for the efficient efflux of substrates into the blood (28), whereas, although spec-

Fig. 3. Growth dependence of \(^{14}\text{C}\)Gly-Sar uptake from the apical (A) and basolateral (B) sides in MDCK cells. MDCK cells cultured for 2–10 days were incubated with 20 \( \mu \text{M} \) \(^{14}\text{C}\)Gly-Sar (apical side, 30 min and pH 6.0; basolateral side, 15 min and pH 7.4) in the absence (○) or presence (●) of 10 mM unlabeled Gly-Sar. Broken lines show the specific \(^{14}\text{C}\)Gly-Sar uptake by subtracting the nonspecific uptake estimated in the presence of 10 mM unlabeled Gly-Sar (●) from the total uptake (○). Each point represents the mean ± SE of 3 monolayers.

Fig. 4. Effects of (Gly)n on \(^{14}\text{C}\)Gly-Sar uptake from the apical (A) and basolateral (B) sides in MDCK cells. MDCK cells were incubated at 37°C with 20 \( \mu \text{M} \) \(^{14}\text{C}\)Gly-Sar added to either the apical (pH 6.0, 15 min) or basolateral side (pH 7.4, 5 min) in the absence (open bar) or presence of 10 mM inhibitors (hatched or solid bars). Each bar represents the mean ± SE of 3 monolayers. ** \( P < 0.01 \), significantly different from control.
The high affinity of the renal basolateral peptide transporter may contribute to the uptake of small peptides from the circulation.

$[^{14}C]$Gly-Sar uptake by the basolateral peptide transporter in MDCK cells was inhibited by di- and tripeptide but not by amino acid and tetrapeptide. This substrate specificity is quite similar to that of the apical H$^+$-peptide cotransporter in MDCK cells. However, both transporters in MDCK cells had different transport characteristics as follows: 1) the transport activity of the basolateral transporter was highly dependent on the culture duration, but that of the apical H$^+$-peptide cotransporter was not so much; 2) pH profiles of $[^{14}C]$Gly-Sar uptake by both transporters were quite different from each other; 3) the basolateral transporter showed higher affinity for $[^{14}C]$Gly-Sar uptake than the apical H$^+$-peptide cotransporter; and 4) the basolateral transporter was more sensitive to PCMBS than the apical H$^+$-peptide cotransporter, whereas the apical H$^+$-peptide cotransporter was more potently inhibited by DEPC compared with the basolateral transporter. These results indicated that the basolateral peptide transporter in MDCK cells was distinct from the apical H$^+$-peptide cotransporter in MDCK cells.

Functional characteristics of renal basolateral transporter described above such as pH profiles of Gly-Sar uptake appeared to be different from PEPT1 and PEPT2 (26–28). The apparent $K_m$ values of Gly-Sar for the renal basolateral peptide transporter and PEPT2 were similar, but no immunostaining was observed in the basolateral membranes of the renal tubular cells when using anti-PEPT2 antibody (25). It is, therefore, suggested that the renal basolateral peptide transporter was different from PEPT1 and PEPT2. Taking all information into consideration, the renal basolateral peptide transporter may be the novel peptide transporter that is functionally different from PEPT1.
kidney. Therefore, the basolateral peptide transporter in the isolated proximal straight tubules of the rabbit demonstrated the transepithelial transport of Gly-Sar in vitro microperfusion technique, Barfuss et al. (4) under the present culture conditions. However, using an in vitro microperfusion technique, Barfuss et al. (4) demonstrated the transepithelial transport of Gly-Sar in the isolated proximal straight tubules of the rabbit kidney. Therefore, the basolateral peptide transporter would be expressed in the proximal tubular cells. The low basolateral transport activity in OK and LLC-PK₁ cells may be due to the culture conditions. It was reported that the expression of apical H⁺-peptide cotransporter (PEPT2) in LLC-PK₁ cells was dependent on culture conditions (33).

Two types of glucose transporters exist in mammals: Na⁺-glucose cotransporters (SGLT1 and SGLT2) and facilitated glucose transporters (GLUT-1 through GLUT-5). Whereas SGLTs are primarily expressed in the brush-border membranes of intestinal and renal epithelial cells, GLUTs are found in plasma (basolateral) membranes of all cells examined so far (9, 11). In addition, it is suggested that the different GLUTs expressions along the nephron segments play unique functional roles for each isoform in renal glucose handling; i.e., GLUTs in the proximal tubular cells contribute to the net transepithelial reabsorption of glucose, whereas GLUTs in the other segments may be important in cell glucose metabolism by accumulating the glucose (9, 12, 30). Similar to GLUTs, it is possible that the basolateral-type peptide transporters are expressed in various tissues and show more functional and molecular diversity.

In conclusion, this is the first demonstration that the peptide transporter is functionally expressed in the basolateral membranes of the kidney. Functional characteristics of this transporter are different from those of known peptide transporters (PEPT1, PEPT2, and the intestinal basolateral peptide transporter). Although further studies are needed to elucidate the physiological roles of this transporter, these findings may provide important information to understand the renal handling of small peptides.

PEPT2, and the intestinal basolateral peptide transporter.

The renal cell lines LLC-PK₁ and OK represent the basic characteristics of proximal tubules, and MDCK displays those of distal tubules or collecting ducts (10). Of these cell lines, MDCK cells had the greatest transport activity of [¹⁴C]Gly-Sar through the basolateral membranes, whereas OK cells had none, and LLC-PK₁ cells had negligible [¹⁴C]Gly-Sar transport activity under the present culture conditions. However, using an in vitro microperfusion technique, Barfuss et al. (4) demonstrated the transepithelial transport of Gly-Sar in the isolated proximal straight tubules of the rabbit kidney. Therefore, the basolateral peptide transporter

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