

Glycylsarcosine Uptake in Rabbit Renal Brush Border Membrane Vesicles Isolated From Outer Cortex or Outer Medulla: Evidence for Heterogeneous Distribution of Oligopeptide Transporters

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ABSTRACT Studies were initially performed in rabbit brush border membrane vesicles (BBMV) prepared from whole cortex plus outer medulla. In these studies using combined tissues, two distinct peptide/H⁺ transport systems were found for glycylsarcosine (GlySar) uptake, with one representing a low-affinity/high-capacity system ($Vm1 = 974$ pmol/mg/10 sec and $Km1 = 4819$ μ M) and the other a high-affinity/low-capacity system ($Vm2 = 220$ pmol/mg/10 sec and $Km2 = 96$ μ M). Thus, under linear conditions, the high-affinity transporter accounted for about 92% of the total transport of dipeptide. To better define the regional heterogeneity of peptide transporter activity in kidney, subsequent studies were performed in vesicles prepared from separately harvested outer cortical and outer medullary tissue. In BBMV studies prepared from outer cortex, two saturable components were revealed for GlySar transport (low-affinity/high-capacity transport system: $Vm1 = 1921$ pmol/mg/10 sec and $Km1 = 11714$ μ M; high-affinity/low-capacity transport system: $Vm2 = 143$ pmol/mg/10 sec and $Km2 = 138$ μ M). However, in BBMV studies prepared from outer medulla, only one saturable component was revealed for GlySar transport (high-affinity/low-capacity transport system: $Vm2 = 168$ pmol/mg/10 sec and $Km2 = 230$ μ M). Overall, these studies support the contention that peptides are handled sequentially in kidney (ie, first by low-affinity transporter PEPT1, and then by high-affinity transporter PEPT2) and that PEPT2 is primarily responsible for the renal reabsorption of peptides and peptidomimetics.

KEYWORDS: Renal peptide heterogeneity, Low-affinity transporter, High-affinity transporter, Glycylsarcosine, Brush border membrane vesicles

INTRODUCTION

Renal peptide transporters play a fundamental role in the reabsorption of peptide-bound amino acids, in the regulation of small peptide plasma concentrations, and in the efficacy and pharmacokinetics of β -lactam antibiotics and other peptidomimetic drugs. In this regard, the transport of oligopeptides has been shown to be proton-dependent, electrogenic and heterogeneous.

As such, the genes encoding two families of H⁺ /peptide cotransporters have been reported in rabbit (1-3), human (4,5), and rat (6-8) along with molecular evidence for tissue specific expression. These investigators and others (9,10) have shown that while PEPT1 is expressed in the intestine and to a smaller extent in kidney, PEPT2 is expressed only in kidney. In addition, more recent studies (11,12) have demonstrated that PEPT1 and PEPT2 are differentially distributed along the proximal tubule, with PEPT1 being predominant in the convoluted segment and PEPT2 being predominant in the straight segment. However, mRNA and/or protein expression levels do not always reflect a similar degree of substrate activity. Thus, the primary objective was to characterize the activity of the high-capacity/low-affinity and low-capacity/high-affinity peptide transporters in brush border membrane vesicles (BBMVs) isolated from different regions of the kidney. A secondary objective was to determine the relative contribution of each transporter in the overall uptake of peptides from renal membrane vesicle preparations.

MATERIALS AND METHODS

Renal Membrane Vesicles

BBMVs were isolated using a divalent cation precipitation method coupled to differential centrifugation, as described previously (13). In these experiments, the vesicles were prepared from whole cortex plus outer medulla of male New Zealand white rabbits (2-3 kg) since both regions possess peptide transport activity (14). Typically, about 12 g of tissue per rabbit were obtained. The entire procedure was carried out on ice or at 4°C. BBMV preparations were prepared the day before use, stored at 4°C overnight and used within 3 days.

Heterogeneity in peptide transporter activity was also assessed using BBMV preparations that were obtained from different regions of rabbit kidney. In this regard, outer cortical and outer medullary tissues were harvested separately from each kidney, as described previously (14-16). Typically, 6 kidneys were used for each preparation (about 2.2 g of tissue from outer cortex and 1.7 g from outer medulla). Once these distinct tissues were harvested, BBMV preparations were processed as described above.

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Marker Enzyme and Protein Assays

Levels of alkaline phosphatase (ALP), a marker for brush border membranes, was determined using a commercially available kit (Sigma Diagnostics, Kit #245, St. Louis, MO). Cross-contamination of BBMV with basolateral membranes was assessed by measuring the Na^+/K^+ -ATPase activity according to Jørgensen and Skou (17) with phosphate determined by the method of Fiske and Subbarow (18). Protein was assayed according to the method of Bradford (19) using γ -globulin as standard.

Uptake Experiments

Uptake studies were conducted by a rapid filtration technique (20) using a 10-place filtering manifold (Hofer Scientific Instruments, San Francisco, CA). All transport measurements were performed at 22°C. Typically, BBMVs were suspended in loading buffer to give a final protein concentration of 5-10 mg/ml and incubated for 1 hour at room temperature before uptake measurements. For studies in the presence of an inwardly directed H^+ gradient, vesicles were suspended in buffer (pH 8.3) containing 50 mM Hepes, 75 mM Tris, and 100 mM K_2SO_4 . Uptake was initiated by mixing 20 μl vesicle suspension with 80 μl uptake medium (pH 6.0) containing varying concentrations of [^{14}C]glycylsarcosine (GlySar; 119 mCi/mmol, Amersham, Chicago, IL) in 50 mM Mes, 50 mM Hepes, 25 mM Tris, and 300 mM mannitol. Under these conditions, the intravesicular pH (pH_{in}) was 8.3 and the final incubation pH (pH_{out}) was 6.7. At 10 seconds, the incubation was terminated by addition of 2 ml ice-cold stop solution (2 mM Hepes and 210 mM KCl, pH 7.5). The content was immediately filtered under vacuum through a prewetted Millipore filter (PHWP, 0.3 μm) and washed five times with 2 ml ice-cold stop solution. The radioactivity remaining on the filters was counted by standard liquid scintillation technique after dissolution in 8 ml scintillation cocktail (Ready Protein, Beckman Instruments, Fullerton, CA). Correction for nonspecific binding was performed by running a zero time in the presence of vesicles where radiolabeled substrate and stop solution were added simultaneously. This value was subtracted from the uptake data. Ganapathy et al (21) have reported that glycylsarcosine does not bind to the membrane vesicles; therefore, radiolabeled GlySar retained on the filters after blank correction represents intravesicular drug. GlySar is also a model substrate due to its resistance to enzymatic hydrolysis in purified BBMV (22).

Uptake studies with D- ^3H glucose (15.0 Ci/mmol, DuPont-NEN, Boston, MA) were performed in a similar manner to that of GlySar with the exception of loading and uptake buffers. In these studies, BBMVs were suspended in a loading buffer containing 100 mM KCl, 100 mM mannitol, and 10 mM Hepes/Tris (pH 7.5). The uptake medium consisted of 125 mM NaCl, 50 mM

mannitol, and 10 mM Hepes/Tris (pH 7.5), and varying concentrations of glucose, giving 100/0 mM for an inwardly directed sodium gradient in the reaction mixture.

Data Analysis

Unless otherwise specified, data are reported as mean \pm SD from at least 3 different membrane preparations, with each preparation conducted in triplicate. Statistical comparisons were performed using analysis of variance (ANOVA; SYSTAT v5.03, Systat, Inc., Evanston, IL). A probability of $P \leq 0.05$ was considered statistically significant. Nonlinear regression analysis was performed using SCIENTIST (version 2.01, MicroMath Scientific Software, Salt Lake City, UT) and a weighting factor of $1/Y$. The quality of the fit was determined by evaluating the coefficient of determination (r^2), the standard error of parameter estimates, and by visual inspection of the residuals.

RESULTS

Uptake of GlySar in BBMV: Cortex plus Outer Medulla Studies

ALP activity for BBMVs isolated from rabbit kidney cortex plus outer medulla was enriched 12.7 ± 1.0 -fold, whereas Na^+/K^+ -ATPase activity was not enriched (0.31 ± 0.09 -fold). These values were consistent with those reported in the literature (23-25) and demonstrate that the BBMV preparations were highly purified with negligible cross-contamination.

Preliminary studies were performed for GlySar using BBMV prepared with combined tissues from rabbit kidney cortex plus outer medulla ($n = 4$). In the presence of an inwardly directed H^+ gradient, initial uptake rates of radiolabeled GlySar (12 μM) were determined as a function of increasing concentrations of unlabeled GlySar (total concentration range of 12-20,000 μM). As determined by nonlinear regression, the data were best fit by a model based on two Michaelis-Menten equations. In this regard, Figure 1 clearly indicates the presence of two distinct peptide/ H^+ transport systems, with one representing a low-affinity, high-capacity system ($V_{\text{m}1} = 974 \pm 204$ pmol/mg/10 sec and $K_{\text{m}1} = 4819 \pm 1596$ μM) and the other a high-affinity, low-capacity system ($V_{\text{m}2} = 220 \pm 20$ pmol/mg/10 sec and $K_{\text{m}2} = 96 \pm 10$ μM). For all analyses, the coefficient of determination (r^2) was ≥ 0.990 . GlySar uptake reflects the activity of peptide transporters alone (ie, PEPT1 and PEPT2). As shown previously (13), cefadroxil was able to completely inhibit the uptake of GlySar, whereas cephalosporins (lacking an α -amino group), amino acids, and organic anions and cations were without effect.

low-capacity type in outer medulla), and are consistent with results published from other laboratories (14-16).

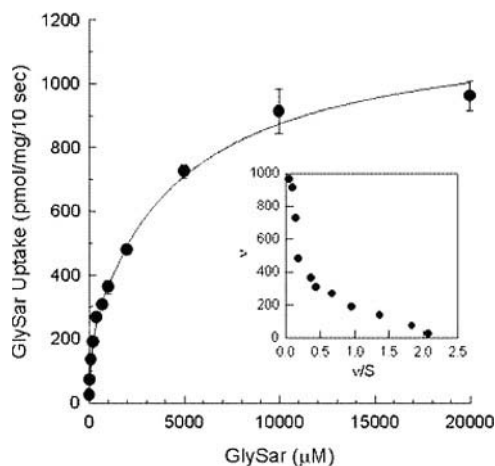


Figure 1 - Saturable uptake of GlySar in BBMVs prepared from whole kidney cortex plus outer medulla of rabbits. Membrane vesicles were suspended in buffer, pH 8.3 (50 mM Hepes, 75 mM Tris and 100 mM K_2SO_4) and uptake was initiated in buffer, pH 6.0 (50 mM Mes, 50 mM Hepes, 25 mM Tris and 300 mM mannitol). The 10-second uptake of 12 μM [^{14}C]GlySar was measured in the presence of increasing concentrations of unlabeled drug. The line was generated using fitted mean parameters (see text) as determined by nonlinear regression analysis. The inset shows a Woolf-Augustinsson-Hofstee transformation of the data [GlySar uptake, ? (pmol/mg/10sec) vs. GlySar uptake/concentration, ?/S ($\mu l/mg/10sec$)]. Data are mean \pm SE from 4 separate membrane preparations.

Uptake of GlySar in BBMVs: Outer Cortex vs. Outer Medulla Studies

For BBMVs isolated from rabbit outer cortex, ALP activity was enriched 14.4 ± 4.3 -fold whereas $Na^+ / K^+ - ATPase$ activity was not enriched (0.65 ± 0.37 -fold). For BBMVs isolated from rabbit outer medulla, ALP and $Na^+ / K^+ - ATPase$ enrichment were 18.4 ± 2.7 -fold and 1.4 ± 0.8 -fold, respectively. These values were consistent with those reported previously (14-16) and demonstrate that the BBMVs preparations were highly purified with negligible cross-contamination. To further validate the method of preparation for BBMVs (outer cortex vs. outer medulla), we examined the uptake of radiolabeled glucose in the presence of an inwardly directed sodium gradient ($n = 7$). In these studies, glucose transport was saturable and the Michaelis-Menten parameters were: $V_m = 5.09$ nmol/mg/10 sec and $K_m = 3840$ μM for outer cortex, and $V_m = 0.47$ nmol/mg/10 sec and $K_m = 323$ μM for outer medulla. These values reflect the heterogeneity of glucose transport in kidney (i.e., low-affinity, high-capacity type in outer cortex; high-affinity,

To better define the heterogeneity of peptide transporter activity in different regions of the kidney, BBMVs preparations were compared using rabbit tissue that had been harvested separately from outer cortex ($n = 5$) and outer medulla ($n = 4$). In a manner analogous to that with combined tissues, the initial uptake rates of radiolabeled GlySar (plus increasing concentrations of unlabeled drug) were studied in the presence of an inwardly directed H^+ gradient. For BBMVs studies prepared from outer cortex, nonlinear regression analysis revealed the presence of two saturable components for GlySar transport (Table 1). One component was consistent with a low-affinity, high-capacity transport system ($V_{m1} = 1921 \pm 647$ pmol/mg/10 sec and $K_{m1} = 11714 \pm 2577$ μM) and the other component was consistent with a high-affinity, low-capacity transport system ($V_{m2} = 143 \pm 54$ pmol/mg/10 sec and $K_{m2} = 138 \pm 56$ μM). However, for BBMVs studies prepared from outer medulla, GlySar transport was best fit by a model based on only one saturable component ($V_{m2} = 168 \pm 68$ pmol/mg/10 sec and $K_{m2} = 230 \pm 132$ μM). For all analyses, the coefficient of determination (r^2) was ≥ 0.985 . A comparison of the saturable uptake components for GlySar in outer cortex vs. outer medulla is displayed in Figure 2.

Table 1 - Transport Parameters of GlySar in BBMVs Isolated From Different Regions of Rabbit Kidney

Region	Outer Cortex	Outer Medulla ^a	Significance ^b
V_{m1} (pmol/mg/10sec)	$1,921 \pm 647$		
K_{m1} (μM)	$11,714 \pm 2,577$		
V_{m2} (pmol/mg/10sec)	143 ± 54	168 ± 68	$p = 0.548$
K_{m2} (μM)	138 ± 56	230 ± 132	$p = 0.196$

^aValues are mean \pm SD from 4-5 separate membrane preparations.

^bP values were determined by ANOVA.

This figure and its corresponding Woolf-Augustinsson-Hofstee plot (Figure 3) clearly show the presence of low- and high-affinity transport systems for GlySar uptake in outer cortex, and a single high-affinity transport system in outer medulla. In fact, when these high-affinity

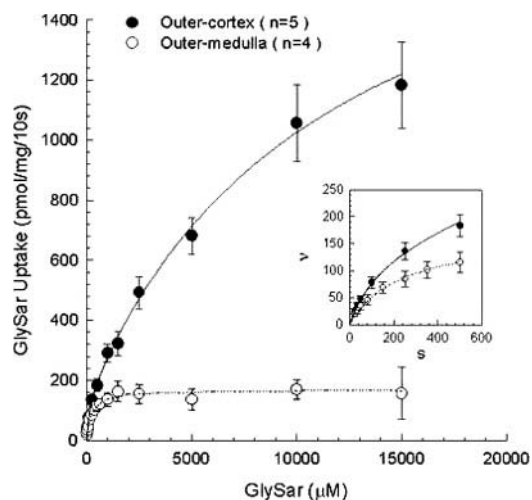


Figure 2 - Saturable uptake of GlySar in BBMVs prepared from outer cortex vs. outer medulla of rabbits. Membrane vesicles were suspended in buffer, pH 8.3 (50 mM Hepes, 75 mM Tris and 100 mM K_2SO_4) and uptake was initiated in buffer, pH 6.0 (50 mM Mes, 50 mM Hepes, 25 mM Tris, and 300 mM mannitol). The 10-second uptake of 25 μM [^{14}C]GlySar was measured in the presence of increasing concentrations of unlabeled drug. The inset highlights the data at low substrate concentrations [GlySar uptake, v (pmol/mg/10sec) vs. GlySar concentration, S (μM)]. Lines were generated using the fitted mean parameters (see Table 1) as determined by nonlinear regression analysis. Data are mean \pm SE from 5 separate membrane preparations for outer cortex and from 4 separate membrane preparations for outer medulla.

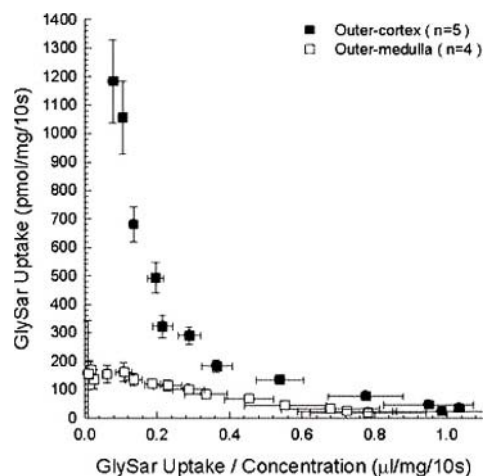


Figure 3 - A Woolf-Augustinsson-Hofstee transformation of the data from Figure 2 [GlySar uptake, v (pmol/mg/10sec) vs. GlySar uptake/concentration, v/S ($\mu l/mg/10sec$)]. Data are mean \pm SE from 5 separate membrane preparations for outer cortex and from 4 separate membrane preparations for outer medulla.

transport parameters were compared, there was no statistical difference in either V_m or K_m (Table 1).

Our results differed from that of Miyamoto et al (14) in their attempt to characterize the sites of peptide transporter activity in rabbit BBMVs prepared from outer cortex and outer medulla. They found that although the affinity of the peptide transport system was similar for GlySar in both membrane preparations (ie, $K_m = 200 \mu M$), the capacity of the system was significantly greater in outer medulla than in outer cortex. However, it should be appreciated that GlySar was examined over a very limited concentration range (ie, 12.5 - 500 μM) in their tissue distribution studies and, as a result, the investigators may have only been able to characterize the high-affinity, low-capacity peptide transporter. In the

present study using a wider range of GlySar concentrations (ie, 25 - 15,000 μM), two saturable transport components were found in BBMVs from outer cortex but only one saturable transport component was found in outer medullary preparations. The two saturable components reflect the low- (K_m 5 - 10 mM) and high-affinity (K_m 100 -200 μM) characteristics of PEPT1 and PEPT2, respectively, and the single saturable component reflects the high-affinity properties of PEPT2. Similar results were also obtained in those studies in which BBMVs were prepared from whole cortex plus outer medulla (ie, mM and μM values of K_m for saturable transporters).

mRNA expression studies in rat (11) have demonstrated that while PEPT1 is specific for early regions of the

proximal tubule (pars convoluta), PEPT2 is overwhelmingly but not exclusively expressed in latter regions of the proximal tubule (pars recta). More recent studies (12) have corroborated these findings and provided definitive evidence for the heterogeneous distribution of PEPT1 and PEPT2 proteins in rat kidney. In this regard, strong PEPT1-specific immunostaining was observed in outer cortex, with progressively weaker staining in deeper cortical regions and no staining in outer medulla. In contrast, strong PEPT2-specific immunostaining was restricted to the outer stripe of outer medulla, which includes the medullary rays protruding into the deeper cortical regions. Our GlySar activity data in rabbit BBMV preparations are consistent with these findings and suggest that peptides are handled in a sequential manner, first by the high-capacity/low-affinity transporter and second by the low-capacity/high-affinity transporter. However, it is appreciated that different mammalian species are being compared, and selective tissue expression may vary in different species.

CONCLUSION

Finally, an important but difficult question to answer involves the contribution of PEPT1 vs. PEPT2 in the overall transport of peptides and peptide-like drugs. In general, many factors could have an influence on transport such as the relative capacities (ie, V_m) and affinities (ie, K_m) of the transporters for a specific substrate, the concentrations of substrate that are present at transporting sites, and the residence times in a particular region during the sequential transport of substrate along the nephron. Using our data in BBMV (whole cortex plus outer medulla) as an example, the transport clearances for the low-affinity (V_m1/K_m1) and high-affinity (V_m2/K_m2) transporters are 0.202 and 2.29 $\mu\text{l}/\text{mg}/10$ sec, respectively. Thus, under linear conditions and perfect mixing (ie, sequential transport not considered), the high-affinity transporter, PEPT2, would account for about 92% of the total GlySar transport. However, this would not always be the case. As shown in Figure 4, while the high-affinity system would dominate at low substrate concentrations (eg, < 500 μM), the low-affinity system would preferentially transport substrate at high concentrations (eg, > 2 mM).

Furthermore, one should realize that this relationship is specific for GlySar and may change for other substrates, and that perfect mixing would not represent the physiological situation. Still, we believe that the high-affinity transporter, PEPT2, is the more important renal peptide carrier given its greater degree of expression (11, 26, 27). Consistent with this tenet are the *in vivo* microinfusion experiments (22) in which glycylsarcosine was substantially reabsorbed from late but not early proximal tubular segments.

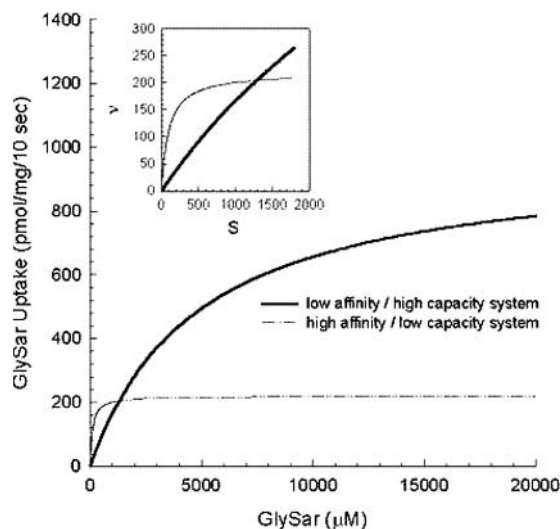


Figure 4 - Contribution of low-affinity, high-capacity transporter and high-affinity, low-capacity transporter on GlySar transport. Data were simulated using the fitted mean parameters of studies in BBMV obtained from whole kidney cortex plus outer medulla ($V_m1 = 974$ pmol/mg/10sec and $K_m1 = 4,819$ μM ; $V_m2 = 220$ pmol/mg/10sec and $K_m2 = 96$ μM).

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REFERENCES

1. Fei Y-J, Kanai Y, Nussberger S, Ganapathy V, Leibach FH, Romero MF, Singh SK, Boron WF, Hediger MA. Expression cloning of a mammalian proton-coupled oligopeptide transporter. *Nature* 1994;368:563-566.
2. Boll M, Markovich D, Weber W-M, Korte H, Daniel H, Murer H. Expression cloning of a cDNA from rabbit small intestine related to proton-coupled transport of peptides, β -lactam antibiotics and ACE-inhibitors. *Pflügers Arch.- Eur. J. Physiol.* 1994;429:146-149.
3. Boll M, Hergert M, Wagener M, Weber WM, Markovich D, Biber J, Clauss W, Murer H, Daniel H. Expression cloning and functional characterization of the kidney cortex high-affinity proton-coupled peptide transporter. *Proc. Natl. Acad. Sci. USA* 1996;93:284-289.
4. Liang R, Fei Y-J, Prasad PD, Ramamoorthy S, Han H, Yang-Feng TL, Hediger MA, Ganapathy V, Leibach FH. Human intestinal H⁺/peptide cotransporter: Cloning, functional expression, and chromosomal localization. *J. Biol. Chem.* 1995;270:6456-6463.
5. Liu W, Liang R, Ramamoorthy S, Fei Y-J, Ganapathy ME, Hediger MA, Ganapathy V, Leibach FH. Molecular cloning of PEPT 2, a new member of the H⁺/peptide cotransporter family, from human kidney. *Biochim. Biophys. Acta* 1995;1235:461-466.

6. Miyamoto K-I, Shiraga T, Morita K, Yamamoto H, Haga H, Taketani Y, Tamai I, Sai Y, Tsuji A, Takeda E. Sequence, tissue distribution and developmental changes in rat intestinal oligopeptide transporter. *Biochim. Biophys. Acta* 1996;1305:34-38.
7. Saito H, Okuda M, Terada T, Sasaki S, Inui K-I. Cloning and characterization of a rat H⁺/peptide cotransporter mediating absorption of β -lactam antibiotics in intestine and kidney. *J. Pharmacol. Exp. Ther.* 1995;275:1631-1637.
8. Saito H, Terada T, Okuda M, Sasaki S, Inui K-I. Molecular cloning and tissue distribution of rat peptide transporter PEPT2. *Biochim. Biophys. Acta* 1996;1280:173-177.
9. Freeman TC, Bentsen BS, Thwaites DT, Simmons NL. H⁺/Dipeptide transporter (PEPT1) expression in the rabbit intestine. *Pflügers Arch. - Eur. J. Physiol.* 1995;430:394-400.
10. Ogihara H, Saito H, Shin B-C, Terada T, Takenoshita S, Nagamachi Y, Inui K-I, Takata K. Immuno-localization of H⁺/peptide cotransporter in rat digestive tract. *Biochem. Biophys. Res. Commun.* 1996;220:848-852.
11. Smith DE, Pavlova A, Berger UV, Hediger MA, Yang T, Huang YG, Schnermann JB. Tubular localization and tissue distribution of peptide transporters in rat kidney. *Pharm. Res.* 1998;15:1244-1249.
12. Shen H, Smith DE, Yang T, Huang YG, Schnermann JB, Brosius FC III. Localization of PEPT1 and PEPT2 proton-coupled oligopeptide transporter mRNA and protein in rat kidney. *Am. J. Physiol.* 1999;276:F658-F665.
13. Akarawut W, Lin C-J, Smith DE. Noncompetitive inhibition of glycylsarcosine transport by quinapril in rabbit renal brush border membrane vesicles: Effect on high-affinity peptide transporter. *J. Pharmacol. Exp. Ther.* 1998;287:684-690.
14. Miyamoto Y, Coone JL, Ganapathy V, Leibach FH. Distribution and properties of the glycylsarcosine-transport system in rabbit renal proximal tubule: Studies with isolated brush-border-membrane vesicles. *Biochem. J.* 1988;249:247-253.
15. Turner RJ, Moran A. Heterogeneity of sodium-dependent D-glucose transport sites along the proximal tubule: Evidence from vesicle studies. *Am. J. Physiol.* 1982;242:F406-F414.
16. Kragh-Hansen U, Røigaard-Petersen H, Jacobsen C, Sheikh MI. Renal transport of neutral amino acids: Tubular localization of Na⁺-dependent phenylalanine- and glucose-transport systems. *Biochem. J.* 1984;220:15-24.
17. Jørgensen PL, Skou, JC. Purification and characterization of (Na⁺-K⁺)-ATPase in pre-REFlections from the outer medulla of rabbit kidney. *Biochim. Biophys. Acta* 1971;233:366-380.
18. Fiske CH, Subbarow Y. The colorimetric determination of phosphorous. *J. Biol. Chem.* 1925;66:375-400.
19. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 1976;72:248-254.
20. Hopfer U, Nelson K, Perrotto J, Isselbacher KJ. Glucose transport in isolated brush-border membrane from rat small intestine. *J. Biol. Chem.* 1973;248:25-32.
21. Ganapathy V, Burckhardt G, Leibach FH. Characteristics of glycylsarcosine transport in rabbit intestinal brush-border membrane vesicles. *J. Biol. Chem.* 1984;259:8954-8959.
22. Silbernagl S, Ganapathy V, Leibach FH. H⁺ gradient-driven dipeptide reabsorption in proximal tubule of rat kidney. Studies in vivo and in vitro. *Am. J. Physiol.* 1987;253:F448-F457.
23. Evers C, Haase W, Murer H, Kinne R. Properties of brush border vesicles isolated from rat kidney cortex by calcium precipitation. *Membrane Biochem.* 1978;1:203-219.
24. Griffiths DA, Hall SD, Sokol PP. Effect of 3'-azido-3'-deoxythymidine (AZT) on organic ion transport in rat renal brush border membrane vesicles. *J. Pharmacol. Exp. Ther.* 1992;260:128-133.
25. McKinney TD, Kunemann ME. Procainamide transport in rabbit renal cortical brush border membrane vesicles. *Am. J. Physiol.* 1985;249:F532-F541.
26. Leibach FH, Ganapathy V. Peptide transporters in the intestine and the kidney. *Annu. Rev. Nutr.* 1996;16:99-119.
27. Daniel H, Herget M. Cellular and molecular mechanisms of renal peptide transport. *Am. J. Physiol.* 1997;273:F1-F8.