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Distribution and properties of the glycylsarcosine-transport system in rabbit renal proximal tubule

Studies with isolated brush-border-membrane vesicles

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The distribution and properties of the peptide-transport system in rabbit renal proximal tubule was examined with glycylsarcosine as the substrate and using brush-border-membrane vesicles derived from pars convoluta (outer cortex) and pars recta (outer medulla). The dipeptide was transported into these vesicles against a concentration gradient in the presence of an inward-directed H^+ gradient, demonstrating the presence of a H^+-coupled peptide-transport system in outer-cortical as well as outer-medullary brush-border membranes. Even though the transport system was electrogenic and was energized by a H^+ gradient in both membranes, the system was more active in outer medullary membranes than in outer cortical membranes. Kinetic analysis showed that, although the affinity of the transport system for glycylsarcosine was similar in both membrane preparations, the capacity of the system was significantly greater in outer medulla than in outer cortex. In addition, the pH profiles of the peptide-transport systems in these membrane preparations also showed dissimilarities. The greater dipeptide uptake in one membrane vis-à-vis the other may probably be due to the difference in the affinity of the transport system for H^+ and/or the difference in peptide/H^+ stoichiometry.

INTRODUCTION

The proximal tubule of the mammalian nephron is anatomically divided into a convoluted part, the pars convoluta, and a part which is less convoluted, known as the pars recta (Kriz & Kaissling, 1985). Structurally, the proximal tubule can be divided into three segments. The first segment (S_1 or P_1) is mainly confined to the pars convoluta, and the third segment (S_3 or P_3) represents the major part of the pars recta. The second segment (S_2 or P_2) comprises the end of pars convoluta and the very beginning of pars recta. Successful development of experimental techniques to study the transport properties in different segments of the proximal tubule, at both cellular and brush-border-membrane levels, has led to numerous investigations, which have shown that the transport systems responsible for reabsorption of glucose and amino acids in different parts of the proximal tubule exhibit different kinetic properties (Barfuss *et al.*, 1980; Barfuss & Schafer, 1981; Turner & Moran, 1982*a*,*b*,*c*; Kragh-Hansen & Sheikh, 1984; Kragh-Hansen *et al.*, 1984; Roigaard-Peterson & Sheikh, 1984; Horio *et al.*, 1986). In general, the transport systems in pars convoluta are of the low-affinity and high-capacity type, whereas the transport systems in pars recta are of high-affinity and low-capacity type. It is well known from micropuncture and microperfusion studies that a large part of the filtered glucose and amino acids is reabsorbed in the early part of the proximal tubule, corresponding mainly to pars convoluta (Silbernagl, 1985). Consequently, the luminal concentrations of these organic solutes are high in pars convoluta, but relatively very low in pars recta. Therefore the different kinetic properties of the glucose- and amino acid-transport system in these two segments ensure maximal reabsorption of these solutes under physiological conditions.

All the transport systems whose distribution and properties in different segments of the proximal tubule have been studied so far represent Na^+-dependent transport mechanisms. We have recently discovered the presence of a transport system that is specific for small peptides in mammalian proximal tubule (Ganapathy & Leibach, 1982, 1986; Ganapathy *et al.*, 1984). This peptide-transport system represents the most recent addition to the array of transport systems already known to exist in mammalian kidney, but it distinguishes itself from the other systems in being energized by a H^+ gradient rather than an Na^+ gradient (Ganapathy & Leibach, 1983; Miyamoto *et al.*, 1985; Takuwa *et al.*, 1985). The purpose of the present work was to investigate the distribution and properties of this novel transport system in brush-border membrane vesicles isolated from pars convoluta (outer cortex) and from pars recta (outer medulla).

EXPERIMENTAL

Materials

[*glycyl*-U-^{14}C]Glycylsarcosine (sp. radioactivity 112 mCi/mmol) was purchased from The Radiochemical Centre, Amersham, Bucks, U.K. D-[1-^{3}H(n)]Glucose (sp. radioactivity 15.5 Ci/mmol) was obtained from New England Nuclear Corp., Boston, MA, U.S.A. Valinomycin and carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone (FCCP) were supplied by Sigma. Glycyl-

Abbreviations used: FCCP, carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone; Gly-Sar, glycylsarcosine.

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proline *p*-nitroanilide was generously given by Professor A. Barth, Martin-Luther-Universität, Halle-Wittenberg, German Democratic Republic.

Methods

Preparation of brush-border membrane vesicles. Brush-border membrane vesicles were prepared from pars convoluta and from pars recta as described by Turner & Moran (1982*a*), with a few modifications. Rabbits of either sex weighing approx. 1–2 kg were killed by CO_2 poisoning. The kidneys were excised and the capsules removed. Outer-cortical tissue was collected by taking slices of about 0.4 mm thick from the surface of the kidney with a Stadie–Riggs microtome. Strips of outer-medullary tissue (about 1–1.5 mm thick) were dissected from the outer strip of outer medulla by using surgical scissors. Both the outer-cortical and the outer-medullary tissues were obtained from the same kidneys. Typically 12 kidneys were used for each preparation. The amount of tissue collected from 12 kidneys was 6.8 ± 0.4 g for outer cortex and 8.0 ± 0.6 g for outer medulla. Turner & Moran (1982*a*) as well as others (Kragh-Hansen & Sheikh, 1984; Kragh-Hansen *et al.*, 1984; Roigaard-Peterson & Sheikh, 1984) used Ca^{2+} as the aggregating cation to prepare brush-border-membrane vesicles from outer cortex and outer medulla. However, owing to a recent report that use of Ca^{2+} results in membrane vesicles that are leaky to Na^+ and other cations (Sabolic & Burckhardt, 1984), we chose to use Mg^{2+} instead of Ca^{2+}. Moreover, the homogenization buffer employed in our procedure contained EGTA to chelate any endogenous Ca^{2+}. The exact protocol for the preparation of brush-border-membrane vesicles using this Mg^{2+}/EGTA precipitation method has been described previously in detail (Miyamoto *et al.*, 1985). The brush-border-membrane vesicles were washed twice in desired preloading buffer and finally suspended in a small volume of the same buffer by passing the membranes through a 25-gauge needle. The protein concentration in these membrane suspensions was adjusted to 10 mg/ml. The membrane suspensions were then divided into small portions and stored in liquid N_2 until use.

Enzyme assays. The purity of the membrane preparations was assessed by measuring the activities of four brush-border marker enzymes: alkaline phosphatase, aminopeptidase N, γ-glutamyl transpeptidase and dipeptidyl peptidase IV. Contamination with other subcellular fractions was monitored by measuring respective marker enzymes: ouabain-sensitive K^+-phosphatase for basal-lateral membranes; acid phosphatase for lysosomes; succinate dehydrogenase for mitochondria; and glucose 6-phosphatase for microsomes (microsomal fractions). Alkaline phosphatase was determined by the liberation of *p*-nitrophenol from *p*-nitrophenyl phosphate in 50 mM-glycine/NaOH buffer, pH 10.5, in the presence of 0.5 mM-$MgCl_2$ (Ganapathy *et al.*, 1985). Acid phosphatase was also determined with *p*-nitrophenyl phosphate as the substrate, but in citrate buffer, pH 5.0 (Pillion *et al.*, 1985). Dipeptidyl peptidase IV was measured with glycylproline *p*-nitroanilide as the chromogenic substrate, by the method of Hama *et al.* (1982). Aminopeptidase N and γ-glutamyl transpeptidase were assayed by using leucine *p*-nitroanilide and γ-glutamic acid *p*-nitroanilide as substrates respectively (Ganapathy *et al.*, 1981*a*). Ouabain-sensitive K^+-phosphatase was determined with *p*-nitrophenyl phosphate as the substrate, as described by Gustin & Goodman (1981). Glucose-6-phosphatase was determined at pH 6.0 in the presence of 5 mM-fluoride (to inhibit protein phosphatases). Succinate dehydrogenase was assayed as described by Pennington (1961).

Uptake measurements. Uptake measurements were made at room temperature (22–23 °C) as described previously (Ganapathy *et al.*, 1981*b*) by using a rapid filtration technique. Millipore filters (DAWP type; pore size 0.65 μm) were used in all cases. Uptake of glycylsarcosine (Gly-Sar) in brush-border-membrane vesicles was determined in the absence of Na^+, but in the presence of an inward-directed H^+ gradient ($[pH]_i = 8.4$; $[pH]_o = 6.7$). Uptake of glucose was determined in the presence of an inward-directed Na^+ gradient ($[Na^+]_o = 120$ mM; $[Na^+]_i = 0$).

RESULTS

Purity of brush-border membrane preparations

The purity of brush-border membranes isolated from outer cortex and from outer medulla was assessed by measuring brush-border marker enzymes as well as marker enzymes for other cellular organelles. The activities of these enzymes in membrane preparations were compared with activities in the initial homogenate in order to calculate enrichment. The data are given in Table 1. Alkaline phosphatase, γ-glutamyl transpeptidase, aminopeptidase N and dipeptidyl peptidase IV were used as markers for brush-border membranes. The activities of these enzymes were enriched 5–6-fold in brush-border membranes isolated from outer cortex and outer medulla. In contrast, ouabain-sensitive K^+-phosphatase, a basal-lateral marker enzyme, was enriched less than 1.6-fold in these preparations. Similarly, the enrichment of marker enzymes for various intracellular organelles such as lysosomes (acid phosphatase), mitochondria (succinate dehydrogenase) and microsomes (glucose-6-phosphatase) was very small (0.8–1.6-fold).

Turner & Moran (1982*a*), in their original paper on the isolation of brush-border-membrane vesicles from the outer cortex and the outer medulla of the rabbit kidney, reported that the activity of alkaline phosphatase was enriched in the final membrane preparations by a factor of 6.3–8.1. In contrast, Kragh-Hansen *et al.* (1984) reported a better enrichment of alkaline phosphatase (8.4–12.2-fold) in their membrane preparations, though they employed a method similar to that of Turner & Moran (1982*a*). The enrichment of alkaline phosphatase in our membrane preparations was slightly less than the above-reported values. We employed the EGTA/Mg^{2+}-precipitation method to isolate the brush-border membranes, instead of the Ca^{2+}-precipitation method used by Turner & Moran (1982*a*) and by Kragh-Hansen *et al.* (1984), and this probably was the reason for the low enrichment of the marker enzymes in the present study. In spite of this, we chose the EGTA/Mg^{2+}-precipitation method to isolate the brush-border membrane vesicles for the investigation of the H^+-gradient-dependent peptide transport, because the membrane vesicles prepared by this method have been shown to be superior to the membrane vesicles prepared by the Ca^{2+}-precipitation method for peptide-transport studies (Takuwa *et al.*, 1985). In the brush-border-membrane vesicles prepared

Table 1. Enzyme activities in brush-border-membrane (BBM) vesicles isolated from outer cortex and from outer medulla

Activities of all enzymes except glucose-6-phosphatase are expressed as μmol/min per mg of protein. Glucose-6-phosphatase activity is expressed as nmol/min per mg of protein. The results are means ± S.E.M. for three to four determinations.

Enzyme	Enzyme activity					
	Outer cortex			Outer medulla		
	Homogenate	BBM	Enrichment	Homogenate	BBM	Enrichment
Alkaline phosphatase	0.164 ± 0.015	0.82 ± 0.07	5.04 ± 0.41	0.106 ± 0.025	0.60 ± 0.10	5.91 ± 0.43
γ-Glutamyl transpeptidase	0.580 ± 0.033	2.75 ± 0.39	4.79 ± 0.73	0.449 ± 0.046	3.29 ± 0.06	8.07 ± 0.87
Aminopeptidase N	0.248 ± 0.011	1.44 ± 0.10	5.85 ± 0.59	0.083 ± 0.010	0.73 ± 0.11	8.73 ± 0.31
Dipeptidyl peptidase IV	0.138 ± 0.017	0.66 ± 0.06	4.97 ± 0.52	0.075 ± 0.026	0.41 ± 0.10	6.00 ± 0.90
Ouabain-sensitive K^+-phosphatase	0.015 ± 0.001	0.017 ± 0.002	1.16 ± 0.05	0.017 ± 0.003	0.027 ± 0.003	1.61 ± 0.14
Acid phosphatase	0.033 ± 0.004	0.052 ± 0.003	1.58 ± 0.09	0.036 ± 0.004	0.056 ± 0.005	1.57 ± 0.02
Succinate dehydrogenase	0.041 ± 0.003	0.057 ± 0.013	1.38 ± 0.31	0.040 ± 0.001	0.051 ± 0.012	1.24 ± 0.27
Glucose-6-phosphatase	7.81 ± 0.58	6.18 ± 0.47	0.80 ± 0.05	4.16 ± 0.22	3.33 ± 0.70	0.79 ± 0.13

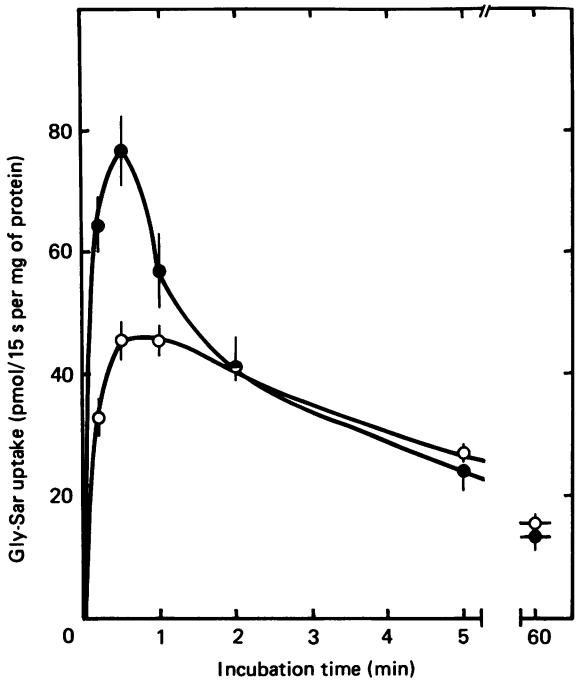

Fig. 1. Gly-Sar uptake in brush-border-membrane vesicles prepared from outer cortex and outer medulla

Membrane vesicles were preloaded with 50 mM-Hepes/75 mM-Tris buffer, pH 8.4, containing 100 mM-K_2SO_4. Uptake was initiated by adding 40 μl of membrane suspension (0.4 mg of protein) to 160 μl of 50 mM-Hepes/50 mM-Mes/25 mM-Tris buffer, pH 5.9, containing 300 mM-mannitol and labelled Gly-Sar. Under these conditions the intravesicular pH was 8.4 and the extravesicular pH was 6.7. The final concentration of Gly-Sar was 15 μM. Results are means ± S.E.M. for four experiments with duplicate determinations. ●, Outer medulla; ○, outer cortex.

in the presence of Ca^{2+}, the experimentally imposed ion gradients have been shown to dissipate at a higher rate than in the brush-border membrane vesicles prepared in the absence of Ca^{2+} (Sabolic & Burckhardt, 1984).

H^+-gradient-driven Gly-Sar uptake in brush-border-membrane vesicles prepared from outer cortex and from outer medulla

Uptake of Gly-Sar into brush-border-membrane vesicles isolated from outer cortex and from outer medulla was studied in the presence of an inward-directed H^+ gradient ($[pH]_i = 8.4$; $[pH]_o = 6.7$). Fig. 1 describes the time course of the uptake in these vesicles. Gly-Sar uptake was energized by the H^+ gradient in brush-border-membrane vesicles prepared from outer cortex as well as in brush-border-membrane vesicles prepared from outer medulla, because, in both cases, there was a transient accumulation of Gly-Sar inside the vesicles above the equilibrium value. However, whereas the equilibrium uptake of the dipeptide was the same in these two preparations of membrane vesicles, the initial uptake rates were higher in brush-border-membrane vesicles from outer medulla than in those from outer cortex. Moreover, the intravesicular concentration of Gly-Sar at the peak of the overshoot was about 6 times the equilibrium value in the outer-medullary-membrane

Table 2. Effect of valinomycin and FCCP on Gly-Sar uptake in brush-border membrane vesicles isolated from outer cortex and from outer medulla

Uptake of Gly-Sar (15 μM) was determined with 15 s incubations. Final concentration of valinomycin (VAL) and FCCP were 10 μM and 20 μM respectively. Stock solutions of these ionophores were prepared in ethanol. The final concentration of ethanol in the incubation medium during uptake was 1.3%. The results are means ± S.E.M. for three determinations. Abbreviation: Addn., addition(s).

Vesicular parameters					Uptake			
Intravesicular		Extravesicular			Outer cortex		Outer medulla	
pH	$[K^+]$ (mM)	pH	$[K^+]$ (mM)	Addn.	(pmol/mg of protein)	(%)	(pmol/mg of protein)	(%)
8.4	200	6.7	40	–	28.7 ± 1.1	100	45.8 ± 0.8	100
8.4	200	6.7	40	VAL	43.2 ± 0.2	151	60.3 ± 0.4	132
8.4	200	6.7	40	FCCP	6.7 ± 1.5	23	6.9 ± 1.0	15
8.4	200	6.7	200	VAL, FCCP	14.1 ± 0.2	14	5.2 ± 1.2	11

vesicles, whereas the corresponding ratio was only about 3 in outer-cortical-membrane vesicles. The equilibrium uptake values suggest that the intravesicular volume per mg of protein was the same in both membrane preparations (0.85 ± 0.04 μl/mg of protein in outer-cortical-membrane vesicles and 0.73 ± 0.09 μl/mg of protein in outer-medullary-membrane vesicles).

Electrogenicity of Gly-Sar uptake in outer-medullary and outer-cortical brush-border-membrane vesicles

Dipeptide–H^+ co-transport has been shown to be electrogenic in brush-border-membrane vesicles isolated from whole cortex (Ganapathy & Leibach, 1983; Miyamoto *et al.*, 1985; Takuwa *et al.*, 1985). We studied the effect of a valinomycin-induced K^+-diffusion potential (inside-negative) on Gly-Sar uptake in outer-medullary and outer-cortical brush-border-membrane vesicles. The data in Table 2 show that, in both membrane preparations, Gly-Sar uptake was stimulated by the inside-negative K^+-diffusion potential generated by valinomycin, demonstrating the electrogenic nature of Gly-Sar transport in outer cortex as well as in outer medulla. FCCP, a proton ionophore, inhibited H^+-gradient-driven Gly-Sar uptake in outer-cortical and in outer-medullary brush-border-membrane vesicles. Since FCCP inhibition was observed in control and in short-circuited membrane vesicles, we conclude that both the generation of an inside-positive H^+-diffusion potential (control vesicles) and the dissipation of the pH gradient (short-circuited vesicles) inhibit Gly-Sar uptake. These characteristics of Gly-Sar uptake were similar in outer-cortical and in outer-medullary membrane vesicles (Table 2).

Effect of extravesicular pH on Gly-Sar uptake

The effect of extravesicular pH (final pH after mixing the membranes with the uptake buffer) on Gly-Sar uptake was examined over the pH range 5.5–8.0 in outer-cortical and outer-medullary brush-border-membrane vesicles. The intravesicular pH in these experiments was 8.4. Fig. 2 shows that the extravesicular pH had a profound effect on Gly-Sar uptake in both membrane preparations. Optimal pH for uptake was found to be 6.0, and the uptake drastically decreased above pH 7.0. However, at all extravesicular pH values studied, except pH 8.0, uptake in outer-medullary brush-border-membrane vesicles was higher than in outer-cortical brush-border-membrane vesicles. At pH 8.0, uptake in both membrane preparations was similar. Decreasing the extravesicular pH from 8.0 to 7.0 caused a 4.8-fold stimulation of uptake in outer-cortical membrane vesicles, whereas, under similar conditions, uptake in outer-medullary membrane vesicles was stimulated 7.9-fold.

Kinetics of Gly-Sar uptake

The dependence of initial rates of Gly-Sar uptake on Gly-Sar concentration was studied over the concentration range 12.5–500 μM in membrane vesicles derived from outer cortex and from outer medulla. In both membrane preparations, Gly-Sar uptake was saturable and followed Michaelis–Menten kinetics. The results are given in Fig. 3 as Eadie–Hofstee plots (v/s against v). The plots were linear in both cases ($r^2 > 0.97$). Kinetic constants (the apparent affinity constant, K_t, and the maximal velocity, $V_{max.}$) were calculated by linear-regression analysis. In these experiments the initial uptake rates were measured with 15 s incubations. Though Gly-Sar uptake was not linear up to 15 s (Fig. 1), this incubation time was selected to reduce the experimental variations which are inherent in uptake-rate determinations with shorter incubations. Accordingly, the kinetic parameters calculated from these experiments represent only approximate values. In outer-cortical brush-border-membrane vesicles, K_t was 0.20 ± 0.01 mM and $V_{max.}$ was 0.39 ± 0.04 nmol/15 s per mg of protein. In outer-medullary brush-border-membrane vesicles, K_t was 0.20 ± 0.01 mM and $V_{max.}$ was 0.72 ± 0.03 nmol/15 s per mg of protein. The data show that, although the affinity of the transport system for Gly-Sar was similar in both membrane preparations, the capacity of the transport system in outer medulla was significantly greater than in outer cortex.

Kinetics of glucose uptake

To validate the method of preparation of outer-cortical and outer-medullary brush-border-membrane vesicles used in the present study, we calculated kinetic parameters for glucose uptake in these membrane preparations and compared these values with data (not

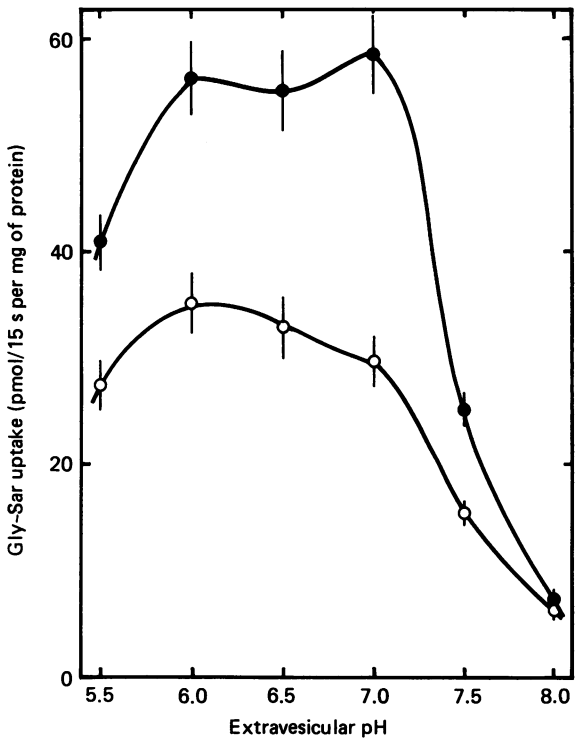

Fig. 2. Effect of extravesicular pH on Gly-Sar uptake in brush-border-membrane vesicles prepared from outer cortex and outer medulla

Membrane vesicles were preloaded with 50 mM-Hepes/75 mM-Tris buffer, pH 8.4, containing 100 mM-K_2SO_4. Initial uptake rates were measured with 15 s incubations over the extravesicular pH range 5.5–8.0. To produce an extravesicular pH of 5.5, 6.0 and 6.5, the uptake buffer contained 100 mM-Mes and differing concentrations of Tris and mannitol ([Tris]+[mannitol] = 325 mM). In the case of extravesicular pH values 7.0, 7.5 and 8.0, the uptake buffer contained 50 mM-Hepes and differing concentrations of Tris and mannitol ([Tris]+[mannitol] = 375 mM). The final concentration of Gly-Sar was 15 μM. Results are means ± S.E.M. for three experiments with duplicate determinations. ●, Outer medulla; ○, outer cortex.

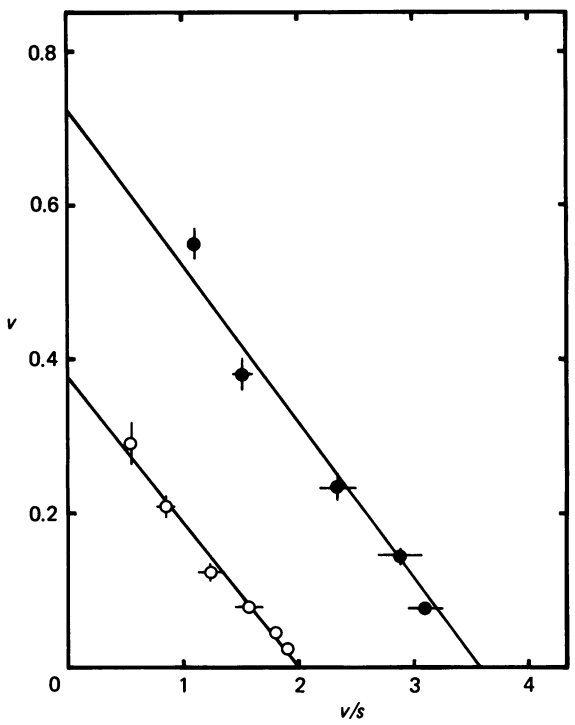

Fig. 3. Kinetics of Gly-Sar uptake

The dependence of initial rates of Gly-Sar uptake on Gly-Sar concentration was studied over a range of 12.5–500 μM in brush-border-membrane vesicles prepared from outer cortex and outer medulla. Initial uptake rates were measured with 15 s incubations in the presence of an inward-directed H^+ gradient ($[pH]_i = 8.4$; $[pH]_o = 6.7$). The data are given as Eadie–Hofstee plots (v/s against v). Each datum point represents the mean ± S.E.M. for three experiments with duplicate determinations. Where the S.E.M. is not shown, it falls within the area of the symbol. v is expressed as nmol/15 s per mg of protein and s (the Gly-Sar concentration) is expressed in mM; ●, outer medulla; ○, outer cortex.

shown) previously published from other laboratories. Glucose uptake was saturable and followed Michaelis–Menten kinetics in outer-cortical as well as in outer-medullary brush-border-membrane vesicles. The uptake system in outer cortex was of the low-affinity and high-capacity type (K_t, 2.25 mM; $V_{max.}$, 5.76 nmol/15 s per mg of protein), whereas the uptake system in outer medulla was of the high-affinity and low-capacity type (K_t, 0.40 mM; $V_{max.}$, 2.30 nmol/15 s per mg of protein). The results published from other laboratories were essentially the same (Turner & Moran, 1982*a*; Kragh-Hansen *et al.*, 1984; Horio *et al.*, 1986).

DISCUSSION

We employed a procedure similar to that of Turner & Moran (1982*a*) to obtain the outer-cortical and outer-medullary tissues from rabbit kidneys. Comparison of our data on glucose uptake with those of Turner & Moran (1982*a*) and of others (Kragh-Hansen *et al.*, 1984; Horio *et al.*, 1986) demonstrates that the membrane preparations employed in our study were, in fact, derived from pars convoluta (outer cortex) and from pars recta (outer medulla).

The results presented here show that the brush-border-membrane vesicles derived from outer cortex as well as from outer medulla are capable of accumulating Gly-Sar against a concentration gradient. The process is H^+-gradient-dependent and electrogenic in both membranes. However, the brush-border-membrane vesicles isolated from outer medulla have a higher capacity to transport Gly-Sar compared with the vesicles from outer cortex. Kinetic analysis, however, shows that the apparent affinity of the transport system for Gly-Sar was similar in both membrane preparations. This is in contrast with the properties of glucose- and amino acid-transport systems. The transport systems for these solutes are of the low-affinity and high-capacity type in outer cortex, but are of the high-affinity and low-capacity type in outer medulla (Barfuss *et al.*, 1980; Barfuss & Schafer, 1981; Turner & Moran, 1982*a*; Kragh-Hansen & Sheikh, 1984; Kragh-Hansen *et al.*, 1984; Roigaard-Peterson & Sheikh, 1984; Horio *et al.*, 1986). It has also been shown

that the Na^+/glucose stoichiometry is 1:1 in outer cortex, but 2:1 in outer medulla (Turner & Moran, 1982*c*; Kragh-Hansen *et al.*, 1984). These differences in the kinetic properties of glucose- and amino acid-transport systems in different segments of the proximal tubule contribute to the maximal reabsorption of these solutes from the filtrate. Since the luminal concentrations of glucose and amino acids under physiological conditions are relatively high in pars convoluta, the low-affinity but high-capacity transport systems for these solutes in this segment of the proximal tubule are capable of efficiently reabsorbing the bulk of these compounds. Owing to this efficient reabsorption, the luminal concentrations of these solutes fall along the proximal tubule. Therefore the later segment, the pars recta, requires high-affinity transport systems for efficient reabsorption of the last traces of these compounds from the tubular lumen.

The situation for di- and tri-peptides is quite opposite to that of glucose and amino acids. The concentration of di- and tri-peptides filtered at the glomerulus is negligible. The filtrate instead contains larger peptides and proteins in significant quantities. These polypeptides are, however, not substrates for the renal peptide-transport system. As these polypeptides pass down the proximal tubule, they may be hydrolysed to generate di- and tri-peptides by the action of various peptidases in the brush-border membrane. The peptidases of renal brush-border membrane represent a very potent set of enzymes capable of degrading a polypeptide to a mixture of amino acids and small peptides. One of these enzymes, dipeptidyl peptidase IV, releases dipeptides from the *N*-terminus of polypeptides in a sequential manner (Miyamoto *et al.*, 1987), and this enzyme represents as much as 4% of the total brush-border membrane protein (Kenny & Booth, 1976). Therefore the concentration of small peptides is low in the beginning of pars convoluta, but it will gradually increase along the tubule and reach significantly higher levels in pars recta. Accordingly, the peptide-transport system is of a low-capacity type in pars convoluta, but is of a high-capacity type in pars recta. These different properties of the peptide-transport system in different segments of the proximal tubule would ensure maximal reabsorption of peptides from the tubular lumen under physiological conditions.

The kinetic properties of the peptide-transport system observed in outer-cortical and outer-medullary brush-border-membrane vesicles in the present study are in agreement with our micropuncture studies on Gly-Sar reabsorption in rat renal tubule (Silbernagl *et al.*, 1986). In these studies the disappearance of Gly-Sar from the perfusion medium was investigated in different segments of rat renal tubule. The disappearance of the dipeptide was 66% if microinfusion took place at early or late sections of pars convoluta, but the disappearance was only 18% if early distal tubule was microinfused. These data indicate that Gly-Sar is mainly reabsorbed from the tubule section between the late proximal and early distal tubule. The proximal straight tubule (pars recta) is the most probable candidate for the site of reabsorption, though the other parts of the loop cannot be excluded. On the other hand, the contribution by pars convoluta to Gly-Sar reabsorption is much smaller compared with the contribution by the later parts of the tubule.

There are at least two possible explanations for the higher Gly-Sar-uptake rates observed in outer-medullary brush-border-membrane vesicles compared with outer-cortical brush-border-membrane vesicles. The former membrane may possess a greater number of peptide-carrier molecules than the latter. This, however, does not appear to be the case, because Gly-Sar uptake was the same at pH 8.0 in both membrane preparations. If the outer-medullary membrane were to contain more carrier molecules, the uptake in this membrane should be higher than in the outer-cortical membrane at all pH values. Alternatively, both membranes contain the same number of carrier molecules, but with different properties, and the carrier system in outer-medullary membrane operates more efficiently than in outer-cortical membrane. Qualitatively, the general characteristics of Gly-Sar uptake, such as H^+-dependence and electrogenicity, are similar in both membranes. However, quantitative aspects, such as affinities of the uptake system for its substrates (peptide and H^+) and peptide/H^+ stoichiometry, may play an important role in determining the efficiency of the uptake system. Our data show that the affinity of the carriers for Gly-Sar is similar in both membranes (Fig. 3). However, the carrier system in outer-medullary membrane is accelerated by a H^+ gradient to a greater extent than that in outer-cortical membrane. Therefore it is likely that the peptide-transport system in outer-medullary brush-border membrane differs significantly, in its affinity for H^+ and/or in its H^+/peptide coupling ratio, from the peptide-transport system in outer-cortical brush-border membrane as is the case with the glucose-transport system (Turner & Moran, 1982*c*; Kragh-Hansen *et al.*, 1984).

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