Transepithelial transport and metabolism of glycine in S1, S2, and S3 cell types of the rabbit proximal tubule

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Parks, Lisa D., and Delon W. Barfuss. Transepithelial transport and metabolism of glycine in S1, S2, and S3 cell types of the rabbit proximal tubule. Am J Physiol Renal Physiol 283: F1208–F1215, 2002; 10.1152/ajprenal.00021.2002.—In the first of two sets of experiments, the lumen-to-cell and cell-to-bath transport rates for glycine were measured in the isolated-perfused medullary pars recta (S3 cells) of the rabbit proximal tubule at multiple luminal glycine concentrations (0–2.0 mM). The lumen-to-cell transport of glycine was saturated, which permitted the calculation of the transport maximum of disappearance rate of glycine from the lumen (pmol-min−1-mm tubular length−1), KM (mM), and paracellular leak (pmol-min−1-mm tubular length−1-mM−1) values for this transport mechanism; these values were 4.3, 0.3, and 0.03, respectively. The cell-to-bath transport did not saturate but showed a linear relationship to cellular glycine concentration, 0.58 pmol-min−1-mm tubular length−1-mM−1. The second set of experiments characterized the transport rate, cellular accumulation, and metabolic rate of lumen-to-cell transported [3H]glycine in all segments (cell types) of the proximal tubule, pars convoluta (S1 cells), cortical pars recta (S2 cells), and medullary pars recta (S3 cells). These proximal tubular segments were isolated and perfused at a single glycine concentration of 11.2 μM. From the results of this study and previous work (Barfuss DW and Schafer JA. Am J Physiol 236: F149–F162, 1979), we conclude that the axial heterogeneity for glycine lumen-to-cell and cell-to-bath transport capacity extends to the medullary pars recta (S3 cells; S1 > S2 < S3 for lumen-to-cell transport and S1 > S2 > S3 for cell-to-bath transport). Also, we conclude that lumen-to-cell transported glycine can be metabolized and its metabolic rate displays axial heterogeneity (S1 > S2 > S3). The physiological significance of these transport and metabolic characteristics of the S3 cell type permits the medullary pars recta to effectively recover glycine from very low luminal glycine concentrations and makes glycine available for protective and maintenance metabolism of the medullary pars recta.

kidney; glutathione; nephron

MUCH IS KNOWN ABOUT THE CHARACTERISTICS of amino acid transport in the epithelial cells of the proximal tubule as determined by several techniques, including whole animal studies, isolated tubule perfusion, microperfusion, cell culture, and membrane vesicle preparations (1, 2, 10, 14, 18, 25). These studies were primarily focused on the transport of the luminal membrane, lumen-to-cell transport. In most of these studies, it is not apparent which of the proximal tubular cell types (S1, S2, or S3) were studied and the metabolic fate of the transported glycine was not determined.

There are two sources of amino acids that are presented to the luminal membrane of the proximal tubule for transport. The first and largest amount is from filtration of blood at the glomerulus, and the second source is the result of digestion of filtered proteins by enzymes located in the brush border. The bulk of the filtered amino acids are removed from the ultrafiltrate by the proximal convoluted tubule (S1 cell type), leaving very little, if any, of the filtered amino acids to be absorbed by the later segments of the proximal tubule, cortical pars recta (S2 cell type), and medullary pars recta (S3 cell type). Free amino acids in the luminal fluid that result from protein digestion are probably absorbed on site or slightly “downstream” of the digestion site. The consequence of the avid lumen-to-cell absorption in the proximal convoluted tubule (S1) is the luminal membrane of the latter segments of the proximal tubule are exposed to very low amino acid concentrations. This implies that these latter segments must acquire the amino acids needed for cellular function from the fluid bathing them, thus the need for transporting amino acids into the tubular epithelium cells at the basolateral membrane. In fact, it has been shown that glycine specifically is avidly transported into the cortical pars recta (S2 cell type) at the basolateral membrane (3).

Because the concentration of amino acids in the luminal fluid of the proximal tubule decreases the farther it flows from the glomerulus, the transport characteristics of the amino acid transport mechanisms need to vary along the proximal tubule to ensure the maximal possible amino acid recovery. Namely, the proximal convoluted tubule (S1) has a much greater transport maximum, lesser affinity, and greater paracellular back-leak (blood-to-lumen) for amino acids than does the cortical proximal straight segment (S2) (3). The latter proximal tubular segments require a much greater affinity and less back-leak to absorb the smaller quantity amino acids from a very low luminal...
amino acid concentration. This pattern of axial heterogeneity of greater transport capacity and lesser affinity in the proximal convoluted tubule compared with a much lower transport capacity, much greater affinity and lesser back-leak in the proximal straight segments is also found for D-glucose absorption (4).

There are several recent reviews of amino acid transport in the kidney and intestine that indicate that the fractional excretion of glycine varies to a greater extent from species to species than do other amino acids (8, 12, 19, 26, 27). Rats and rabbits have a low urinary excretion coupled with a relatively low absorption rate in the proximal tubule (27). This leads to the conclusion that glycine is absorbed beyond the proximal convoluted tubule. It has also been shown that glycine uptake is Na+ dependent in studies using rabbit renal brush-border membrane vesicles (14) and rat canalicular liver plasma membrane vesicles (18).

The metabolic fate of cellular glycine in the proximal tubular cells is very diversified. In addition to being incorporated into structural and enzymatic proteins and used as an energy source, it is involved in protecting the cell against oxidative and osmotic stress. Glycine participates in protection against oxidative stress by two phenomena. One is the protective effect neutral amino acids, glycine being the most effective, affords renal tissue during anoxic episodes, such as periods of nonperfusion followed by reperfusion of blood (29). The mechanism for this protection has not been definitively determined. The second is that glycine is incorporated into the tri-amino acid GSH (20). GSH is the most abundant antioxidant in cells (5–10 mM). These protective effects of glycine are useful in all segments of the proximal tubule, but the medullary pars recta (S3) is the segment that is exposed to the greatest degree of oxidative and osmotic stress. Because it is located in the outer stripe of the outer medulla, the medullary pars recta (S3) can be subjected to a hypertonic environment during periods of water conservation. Under this condition, one mechanism tubular epithelial cells utilize to maintain cellular volume is to increase their intracellular osmolality by synthesizing organic osmolytes to counteract the extracellular hypertonicity (15). One of these organic osmolytes, betaine (N-trimethylglycine), is synthesized from glycine.

The first part of this study was designed to measure both lumen-to-cell and cell-to-bath transport characteristics for glycine in the medullary pars recta segment (S3), determining the values for transport maximum of the disappearance rate of glycine from the lumen (d_{\text{max}}), K_m, and the nonsaturable coefficient (k; presumably paracellular leak). In a second series of experiments, we compared the cellular accumulation, extent of metabolism, and lumen-to-cell and cell-to-bath transport rates of [3H]glycine in the S1, S2, and S3 cell types of the proximal tubule.

**MATERIALS AND METHODS**

Perfusing solutions containing [3H]glycine used in the metabolic experiments of this study were prepared fresh for each experiment. The [3H]glycine had a specific activity of 35.1 Ci/mmol and was stored in ethanol/water (2.98 vol/vol) at 5°C. For perfusion experiments, 20 μl of the [3H]glycine stock solution together with 10 μl of [14C]polyethylene glycol stock solution were dired by a stream nitrogen gas and subsequently reconstituted into 50 μl of artificial perfusion media. The final concentration of [3H]glycine was 11.2 μM. This low glycine concentration was selected to optimize the possibility of measuring any differences in metabolic rate among the three proximal tubule segments (cell types). As determined by HPLC analysis, 96.45 ± 1.27% of the H was associated with the glycine peak in the glycine stock solution. [14C]Polyethylene glycol (11.0 mCi/g) was present in the luminal perfusion solution (2 mM) as a volume marker in all perfusion experiments. All isotopes were purchased from New England Nuclear (Boston, MA). All other chemicals were purchased from Sigma (St. Louis, MO).

Perfusions solutions used in experiments to measure the Michaelis-Menten kinetics of the transport of glycine from the luminal fluid in the S3 segment were the same as described above and below but with the addition of nonradio-labeled glycine, resulting in the following final total glycine concentrations: 0.0112, 0.0885, 0.177, 0.354, 1.000, and 2.000 mM. A minimum of five tubules were perfused at each of these concentrations. In these experiments, the volume marker was L-[3H]glucose (14.6 Ci/mmol) and the tracer glycine was [14C]glycine (112.7 mCi/mmol).

For perfusion and bathing solutions, which contained (in mM) 145 Na+, 140 Cl–, 5.0 K+, 2.5 Ca2+, 1.2 Mg2+, 1.2 SO42–, 2.0 HPO42–/H2PO4–, 0.5 l-glutamate, and 1.0 D-glucose. Osmolality was adjusted to 290 mosmol/kg H2O of water and pH to 7.4. The vital dye FD&C green (250 nM) was present in the perfusion solution only. A sucrose-phosphate buffer solution (4°C) was used for tubule dissection. The buffer solution consisted of (in mM) 125 sucrose, 13.3 NaH2PO4, and 56 Na2HPO4. The final pH was 7.40 (adjusted with 1.0 M NaOH), and the osmolality was adjusted to 290 mosmol/kg H2O by adding water or sucrose (21).

Female New Zealand White, specific pathogen-free rabbits were purchased from Myrtle’s Rabbitry (Thompson Station, TN). All rabbits were maintained on regular rabbit chow and given water ad libitum. Rabbits were anesthetized with ketamine (Ketastat 50 mg/ml), xylazine (100 mg/ml), and acepromazine maleate (1:1:1) and appearance of [3H]glycine in the bathing solution (d_{\text{bath}}), fmol·min⁻¹·mm tubular length⁻¹ and appearance of [3H]glycine in the bathing solution (d_{\text{bath}}), fmol·min⁻¹·mm tubular length⁻¹, and its accumulation in the cells of the tubular segments (μM). Individual segments were dissected manually from coronal sections (7, 9, 13) and identified as described in Barfuss et al. (2) while the segments were bathed in the phosphate-sucrose buffer solution.
At the end of each experiment, the perfused tubular segment was harvested with forceps and placed in 10 μl of a 3% TCA solution. This TCA solution, which contained the cytoplasmic extract, was analyzed for [3H]glycine and [3H]GSH or [3H]GSSG.

Calculations. All calculations to determine \( J_{D, \text{Gly}} \), \( J_{A, \text{Gly}} \), and cell concentration are the same as calculations previously published (2, 3, 6). Cell volume \( (C_V) \) was calculated as in previous studies (3) from tubular dimensions as shown in the equation

\[
C_V = 0.7(r_o^3 - r_i^3)\pi L
\]

in which \( r_o \) and \( r_i \) are the outside and inside radii, respectively, \( L \) is the tubule length, and 0.7 is an empirically derived proportionality factor that accounts for the nonwater volume of the tubule (3).

Mean luminal concentration of glycine ([Gly]_{ML}) was calculated as the arithmetic mean of the perfusate and collectate concentrations. This calculation is justified if the collectate glycine concentration is >40% of the perfusate concentration (4).

The metabolic rate of the transported glycine, except the perfusate, bath, and collectate fluid in the medullary pars recta (S3) segment only, were measured parameters (8). For the determination of the transport of luminal glycine in the medullary pars recta (S3) segment and each experimental condition. Chromatography experiments, de-

Mathematical analysis of chromatographic data. For each HPLC analysis of a sample, all individual chromatographic peaks for GSH, GSSG, glycine, and cysteine-glycine were separated using the following gradient.

The mobile phase was made of eluant A, a 0.05 M sodium acetate solution at pH 4.6, and eluant B, 100% HPLC grade methanol. DNS-glycine, DNS-GSH, DNS-GSSG, and DNS-cysteine-glycine were separated using the following gradient. The gradient program was linear from 0 to 36% eluant B in 6 min. From 6 to 15 min, eluant B continued to run at 36%. From 15 to 21 min, there was a linear gradient from 36 to 60% eluant B that remained constant until 22 min. From 22 to 32 min, the gradient was again linear from 60 to 100% eluant B. Then there was a continuation of 100% eluant B for 5 min to clean the column, followed by a 10-min equilibration period with 100% eluant A to restore initial conditions before a new sample was injected. This procedure generated sufficient fluorescent signals to produce clear chromatographic peaks for GSH, GSSG, glycine, and cysteine-glycine.

Purchased dansylated standards of glycine, cystine, and glutamate eluted ±10 s of the dansylated samples prepared in the laboratory. When tested for yield of dansylated [3H]glycine stock isotope, 96–98% of the [3H] was recovered in the chromatographic peak associated with commercially available DNS-glycine. Dansylated standards for GSH, GSSG, and cysteine-glycine were commercially available and were made and tested in the laboratory. When [3H]GSH was dansylated in the laboratory, 85–90% of the [3H] was associated with the DNS-GSSG peak. The peak associated with cysteine-glycine was not tested with [3H], but in HPLC runs of collectate, bath, and tubule extracts, no significant [3H] was associated with that peak.

Confirmation of steady state. After an individual tubule was warmed and perfused, a period of 15 min was allowed for the attainment of steady state. Steady-state conditions were confirmed by samples for 30 min after the warm-up period (15 min) and constant values for all measured parameters. Consequently, for each tubule, samples were collected during the 30–45 min after the 15-min warm-up period.

Mathematical analysis of chromatographic data. For each HPLC analysis of a sample, all individual chromatographic peak and valley effluents were collected separately and counted for the total amount of [3H] each contained. The amount of [3H] associated with the glycine chromatographic peak was divided by the total amount of [3H] in the sample, giving the fraction of the total sample [3H] that was associated with glycine. This fraction was used to correct the corresponding samples that were used for calculation of the measured parameters \( J_{D, \text{Gly}} \), \( J_{A, \text{Gly}} \), and cellular concentration of glycine.

Statistics. For the determination of the \( J_{\text{max}}, K_m, \) and \( k \) for transport of luminal glycine in the medullary pars recta (S3) only, the data were fitted to the model

\[
\text{Flux}_{\text{Gly}} = (J_{\text{max}} \times [\text{Gly}]_{\text{BK}} + (\text{Gly})_{\text{BK}} + (\text{Gly})_{\text{BK}} \times k)
\]

in which \( \text{Flux}_{\text{Gly}} \) is the \( J_{D, \text{Gly}} \) or \( J_{A, \text{Gly}} \). The mathematical fitting was performed by Hooke-Jeeves and quasi-Newton nonlinear estimation with an (observed – predicted)² loss function using Statistica software.

To determine any significant differences in the metabolic and transport rates of glycine among the three proximal tubule segments (S1–S3) perfused with artificial perfusion media containing 11.2 μM glycine, a minimum of five tubules were perfused for each experimental condition. Three or more flux measurements per tubule were measured and averaged. The mean values from individual tubules were used to compute an overall mean and standard error for each segment and each experimental condition. Chromatography samples were analyzed in duplicate. All data for metabolic and transport rates of glycine were entered into Statistica for
mean and standard error calculations, graphing, and two-way ANOVA and Tukey’s honest significant difference testing \((P < 0.05)\) to determine whether the differences observed among the groups were statistically significant.

**RESULTS**

Characterization of glycine transport. Transport rate of glycine from the luminal fluid and \(J_{A,Gly}\) showed a curvilinear dependency on the \([Gly]_{ML}\). This relationship, shown in Fig. 1, appears to saturate and fits a previously developed model \((Eq. 1)\) for a combination of active (transcellular) and passive (paracellular) transepithelial transport of solutes by the proximal tubular epithelium. Using this model gave estimates for \(J_{max}\), \(K_m\), and \(k\) for both \(J_{D,Gly}\) and \(J_{A,Gly}\). There was no significant difference between the respective values of these three parameters for \(J_{D,Gly}\) and \(J_{A,Gly}\), so for simplicity only the \(J_{D,Gly}\) data are shown (Table 1) and plotted (Fig. 1, line plot).

The cellular concentration of glycine, accumulated from the luminal fluid during luminal transport experiments, was inversely and exponentially proportional to \([Gly]_{ML}\) (Fig. 2). The cell-to-lumen glycine concentration ratio ranged from as low as 4 when \([Gly]_{ML}\) was 1.6 to as high as 36 when \([Gly]_{ML}\) was 0.01 mM.

The \(J_{A,Gly}\) was linearly proportional to the cell glycine concentration (Fig. 3). This relationship did not appear to saturate. It is assumed that all \(J_{A,Gly}\) is transepithelial transport because the paracellular rate was predicted to be not statistically different from 0 \((0.03 \pm 0.09)\).

**Glycine metabolism.** The primary data from the glycine metabolism experiments of this study are summarized in Table 2 and Figs. 4 and 5.

In the convoluted (S1) segment, \(J_{D,Gly}\) was 25.8 and \(J_{A,Gly}\) was 18.9 fmol·min\(^{-1}\)·mm tubule length\(^{-1}\), both greater than the respective values in the cortical pars recta (S2) and medulla pars recta (S3) segments (Table 2). The cellular concentration of \(^{3}H\)glycine in the convoluted (S1) segment was 32.4 \(\mu\)M (less than the S2 and S3 cell types) and \([Gly]_{ML}\) was 6.7 \(\pm\) 0.6 \(\mu\)M. The apparent metabolic rate of the \(^{3}H\)glycine transported into the cells at the luminal membrane of convoluted (S1) segment was 6.9 fmol·min\(^{-1}\)·mm tubule length\(^{-1}\) (Fig. 5). This was greater than the cortical pars recta (S2) (not significant at <0.05) and medullary pars recta (S3) metabolic rates for glycine.

In the cortical pars recta (S2) segment, \(J_{D,Gly}\) was 16.0 and \(J_{A,Gly}\) was 11.2 fmol·min\(^{-1}\)·mm tubule

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Table 1. Summary of estimated \(J_{max}\), \(K_m\), and \(k\) values for medullary pars recta (S3) segment of rabbit proximal tubule for glycine transport

<table>
<thead>
<tr>
<th>Tubule Segment</th>
<th>(J_{max}), (\text{pmol} \cdot \text{min}^{-1} \cdot \text{mm}^{-1})</th>
<th>Affinity (K_m), (\text{mM})</th>
<th>(k), (\text{pmol} \cdot \text{min}^{-1} \cdot \text{mm}^{-1} \cdot \text{mM}^{-1})</th>
<th>Tubule Length, (\text{mm})</th>
<th>(J_{A,Gly}), (\text{fmol} \cdot \text{min}^{-1} \cdot \text{mm}^{-1})</th>
<th>Perfusion Rate, (\text{nl/min})</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>28.5 ± 8.0(^{a})</td>
<td>11.8 ± 7.80(^{a})</td>
<td>1.00 ± 0.32(^{a})</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>S2</td>
<td>2.5 ± 0.4(^{a})</td>
<td>0.70 ± 0.50(^{a})</td>
<td>0.20 ± 0.03(^{a})</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>S3</td>
<td>4.3 ± 0.2(^{a})</td>
<td>0.27 ± 0.04(^{a})</td>
<td>0.03 ± 0.09(^{a})</td>
<td>0.96 ± 0.02</td>
<td>5.3 ± 0.43</td>
<td>7.45 ± 0.09</td>
</tr>
</tbody>
</table>

Values are means ± SE, with each group comprising at least 5 tubules. Previously published estimates of maximum transport \((J_{max})\), \(K_m\), and nonsaturable coefficient \((k)\) for the convoluted (S1) and cortical pars recta (S2) segments have been included for comparison purposes \((3)\). \(J_{A,Gly}\), \(^{1}C\)glucose appearance into the bath solution, NS, not shown. \(^{a}\) \(P < 0.05\), significantly different between values for the same parameter.
The cellular route is the passive diffusion of the amino acid through the tight junction down its concentration gradient. This paracellular transport can be bidirectional from lumen-to-bath or bath-to-lumen, depending on the direction of the chemical gradient of the amino acid. In the isolated perfused tubules in this study, the glycine transport via the paracellular route was from lumen-to-bath because of the outwardly directed concentration gradient, but in vivo glycine concentration gradient is assumed to be from the peritubular fluid to the lumen. Thus the amino acids back-leak into the luminal fluid from the blood. Too great of a blood-to-lumen paracellular leak (back-leak) would make the absorption of amino acids in the medullary pars recta (S3) segment an inefficient process. This futile cycle is prevented by much less paracellular blood-to-lumen flux, as reflected in the lower \( k \) values of the nonsaturable flux in the latter segments of the proximal tubule (Table 1). In the medullary pars recta (S3) segment, the \( k \) value is not statistically different from 0, which greatly prevents glycine from leaking into the luminal fluid in vivo. This optimizes the absorption of glycine in the medullary pars recta (S3) segment. In addition, the paracellular leak for D-glucose is very low in the medullary pars recta (S3) segment compared with the convoluted (S1) and cortical pars recta (S2) segments, again indicating the need to minimize the

**DISCUSSION**

**Glycine transport characteristics in the medullary pars recta (S3) segment.** The transport data of glycine in the medullary pars recta (S3) segment of the proximal tubule measured in this study support the typical two-route model for absorption and transepithelial transport of amino acids, namely, separate paracellular and cellular routes. The cellular route consists of the amino acid being actively transported at the luminal membrane into the cytoplasm and then passively diffusing down its chemical gradient across the basolateral membrane into the peritubular fluid. The paracellular route is prevented by much less paracellular blood-to-lumen flux, as reflected in the lower \( k \) values of the nonsaturable flux in the latter segments of the proximal tubule (Table 1). In the medullary pars recta (S3) segment, the \( k \) value is not statistically different from 0, which greatly prevents glycine from leaking into the luminal fluid in vivo. This optimizes the absorption of glycine in the medullary pars recta (S3) segment. In addition, the paracellular leak for D-glucose is very low in the medullary pars recta (S3) segment compared with the convoluted (S1) and cortical pars recta (S2) segments, again indicating the need to minimize the

**Table 2. Summary of transport of glycine and polyethylene glycol (leak marker) and perfusion conditions in isolated S1–S3 segments of the proximal tubule of the rabbit perfused with 11.2 \( \mu \)M \(^3\text{H}\)glycine**

<table>
<thead>
<tr>
<th>Tubule Segment</th>
<th>Cell Type</th>
<th>( J_{D, Gly} ), fmol·min(^{-1})·mm(^{-1})</th>
<th>( J_{A, Gly} ), fmol·min(^{-1})·mm(^{-1})</th>
<th>Leak, nl·min(^{-1})·mm(^{-1})</th>
<th>Tubule Length, mm</th>
<th>Perfusion Rate, nl·min(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td></td>
<td>25.77 ± 2.99*</td>
<td>18.87 ± 3.27*</td>
<td>0.18 ± 0.006</td>
<td>0.6 ± 0.01</td>
<td>5.83 ± 0.54</td>
</tr>
<tr>
<td>S2</td>
<td></td>
<td>15.98 ± 3.45*</td>
<td>11.18 ± 2.37*</td>
<td>0.13 ± 0.04</td>
<td>0.8 ± 0.02</td>
<td>6.06 ± 0.62</td>
</tr>
<tr>
<td>S3</td>
<td></td>
<td>17.81 ± 1.64*</td>
<td>14.77 ± 0.76*</td>
<td>0.12 ± 0.011</td>
<td>0.7 ± 0.03</td>
<td>5.61 ± 0.42</td>
</tr>
</tbody>
</table>

Values are means ± SE, with each group comprising at least 5 tubules. *\( P < 0.05 \), significantly different between values in the same tubule segment.
back-leak of any absorbed substance (D-glucose, glycine, etc.) for its efficient recovery.

The transport of glycine from the luminal fluid at the luminal membrane of the medullary pars recta (S3) appears to have a greater affinity (lower $K_m$) and a slightly greater $J_{max}$ than does the cortical pars recta (S2) segment of the proximal tubule (Table 1). This greater affinity of the transport mechanism for glycine permits the S3 cell types of the medullary pars recta to recover glycine against a greater lumen-to-cell concentration gradient than can the S1 or S2 cell types of the convoluted and cortical pars recta segments, respectively, of the proximal tubule. This axial location of the S3 cell types in the medullary pars recta segment is ideal for recovery of filtered glycine or glycine from enzymatic digestion of proteins and peptides occurring in all proximal tubular segments. The fact that $J_{max}$ is greater in the medullary pars recta (S3) than in the cortical pars recta (S2) segment may reflect the need for greater transport capacity in the medullary pars recta (S3) segment because of an increased load of glycine from digestion of proteins and peptides “upstream” in the brush border of the cortical pars recta (S2) segment. This same pattern of the medullary pars recta (S3) segment having a greater $J_{max}$ and lesser $K_m$ than the cortical pars recta (S2) segment is found for the transport of L-arginine (unpublished observations). However, this pattern of a greater $J_{max}$ in the medullary pars recta (S3) segment than in the cortical pars recta (S2) segment is not observed for D-glucose (4).

This may reflect the very small load of D-glucose normally delivered to the medullary pars recta (S3) segment, because there is no addition of D-glucose to the luminal fluid as a result of upstream digestion of D-glucose-containing compounds and all filtered D-glucose had been absorbed upstream to the medullary pars recta. Finally, of all the proximal tubular segments, the convoluted (S1) segment has the greatest $J_{max}$ and $K_m$ for glycine and D-glucose (4), reflecting the need to transport large amounts of filtered glycine and D-glucose against a lesser gradient to minimize energy consumption for the recovery of the large filtered loads of these two compounds. This may be the general pattern of transport characteristics for all substances that are filtered and completely absorbed in the proximal tubule: greater transport capacity with lesser affinity in the early (convoluted) proximal tubule with much lesser transport capacity accompanied by greater affinity in the later (cortical and medullary pars recta) segments. To accompany this general pattern of transport characteristic along the proximal tubule with progressively decreasing leakiness of the tight junctions along the proximal tubule results in an effective process for completely recovering filtered substances that have nutritional value.

Evidence that the transport of glycine from the luminal fluid to the cytoplasm in the medulla pars recta (S3) segment is an active process is indicated by the cell-to-lumen glycine concentration ratio, which is much greater than unity at all luminal glycine concentrations studied (Fig. 2). As shown in Fig. 2, the cell-to-lumen ratio exponentially increases as [Gly]$_{ML}$ decreases, indicative of an active transport process(es). In addition, the cellular glycine concentrations from the metabolic experiments (Table 2) indicate active luminal transport of glycine in the medullary pars recta (S3) as well as in the convoluted (S1) and cortical pars recta (S2) segments. This is indicated by cellular glycine concentrations significantly greater than [Gly]$_{ML}$.

Cell-to-bathing fluid glycine transport across the basolateral membrane of the medulla pars recta (S3) segment shows no indication of a saturable process over the range of glycine concentrations examined (Fig. 3). The cell-to-bath glycine transport mechanism at the basolateral membrane may have a very low affinity with a relatively limited transport capacity. This is indicated by the greater cellular glycine levels observed in the medullary pars recta (S3) segment than observed in the convoluted (S1) and cortical pars recta (S2) segments, while these latter segments had the same or greater level of transeellular glycine transport compared with the medullary pars recta (S3) segment (Fig. 4 and Table 2).

Because the medullary region of the kidney is vulnerable to ischemia and is hypertonic, it could be speculated that the much greater cellular levels of glycine in the medullary pars recta (S3) segment could be important in protection against the toxic effects of hypoxia (29) and/or aiding in the regulation of cell volume. The protective effect of neutral amino acids, glycine being the most effective, against the toxic effects of hypoxia is well documented (29). The medullary region of the kidney is more vulnerable to hypoxia because of lesser blood flow than in the kidney cortex. The much greater cellular levels of glycine in the S3 cell types of the medullary pars recta could be important in proving it protection. This may be more impor-

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Fig. 5. Comparison of the metabolic rate of [3H]glycine (11.2 $\mu$M) perfused through the lumen of the convoluted (S1), cortical pars recta (S2), and medullary pars recta (S3) segments of the proximal tubule of the rabbit. *$P < 0.05$, significant difference between the S1 and S3 segments.
Glycine transport/metabolism in medullary pars recta

Determined the percentage of the transported $[^{3}H]$glycine (S3) segment. HPLC analysis enabled us to determine the percentage of the transported $[^{3}H]$glycine that remained in the cells and appeared in the bathing solution in the form of $[^{3}H]$glycine. The analysis of the perfusion samples indicated 95–98% of the $^{3}H$ associated with the $[^{3}H]$glycine chromatographic peak. Therefore, it was assumed that the $[^{3}H]$radiolabel was entering the lumen bound to glycine only. In the cellular samples, the majority (70–85%) of the radiolabel was still present as $[^{3}H]$glycine, but $[^{3}H]$metabolites of glycine were registered as well. Most notably, a significant percentage (8–10%) of the $[^{3}H]$radiolabel in the convoluted (S1) segments was recovered under the chromatographic peak corresponding to GSH (20). $[^{3}H]$GSH was present in the cellular and luminal samples, implying that GSH, and possibly other peptides, could be synthesized and secreted into the lumen (20). The $[^{3}H]$ that appeared in the bathing solution samples was predominantly (95–97%) associated with the glycine HPLC peak. This indicates that at the luminal glycine concentration of 11.2 $\mu$M, most of the metabolites of the luminally absorbed glycine are not transported across the basolateral membrane in the proximal tubule (cell-to-bathing solution).

In conclusion, we determined that glycine transport from the luminal solution is an active process in the medullary pars recta (S3) segment of the proximal tubule and confirms the presence of axial heterogeneity along the proximal tubule for active glycine transport (S1 > S2 > S3). In addition, cellular accumulation of glycine also exhibits axial heterogeneity (S1 < S2 < S3). This latter axial heterogeneity of cellular accumulation of glycine could indicate a diminished transport capacity in basolateral membranes of segments more distant from the glomerulus. Certainly, a less cell-to-bath transport capacity of the basolateral membrane was found in the medullary pars recta (S3) segment. Also, the back-leak of glycine in the medullary pars recta (S3) is practically nonexistent because of the relative impermeability of the tight junctions to glycine in this segment of the proximal tubule. In addition, it is clear that glycine, which is transported into the cell across the luminal membrane, is available to enter into a variety of cellular metabolic pathways in all segments of the proximal tubule. This glycine metabolic rate exhibited axial heterogeneity, with the segments of the proximal tubule that are closest to the glomerulus having the greatest metabolic rates (S1 > S2 > S3).

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