Luminal and Basolateral Membrane Transport of Glutathione in Isolated Perfused S_1, S_2, and S_3 Segments of the Rabbit Proximal Tubule

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Abstract. Lumen-to-bath and bath-to-lumen transport rates of glutathione (GSH) were measured in isolated perfused S_1, S_2, and S_3 segments of the rabbit proximal tubule. In lumen-to-bath experiments, the perfusion solution contained 4.6 μM ^{3}H-GSH with or without 1.0 mM acivicin. In all three segments perfused without acivicin, luminal disappearance rate (J_{DL}) and bath appearance rate (J_{AB}) of ^{3}H-GSH were 14.5 $\pm$ 0.5 and 2.2 $\pm$ 0.8 fmol/min per mm tubule length, respectively. With acivicin present, J_{DL} and J_{AB} were reduced to 1.3 $\pm$ 0.4 and 0.5 $\pm$ 0.3, respectively, with no differences among segments. Cellular concentrations of ^{3}H-GSH in S_1, S_2, and S_3 segments when acivicin was absent were 23.1 $\pm$ 2.0, 31.7 $\pm$ 11.4, and 143.5 $\pm$ 17.9 μM, respectively. With acivicin in perfusate, cellular concentrations were reduced but there was no change in the heterogeneity profile. In bath-to-lumen transport experiments (S_2 segments only), the bathing solution contained 2.3 μM ^{3}H-GSH. ^{3}H-GSH appearance in the lumen (J_{AL}, fmol/min per mm) and cellular accumulation from the bath were studied with and without acivicin in the perfusate. J_{AL} values were 3.0 $\pm$ 0.2 and 0.2 $\pm$ 0.03 while cellular concentrations were 9.5 $\pm$ 1.0 and 6.1 $\pm$ 0.5 μM, respectively. It is concluded that: (*1*) GSH is primarily removed from the luminal fluid after degradation to glycine, cysteine, and glutamate, which are absorbed; (*2*) GSH can be absorbed intact at the luminal membrane; (*3*) the S_3 segment has the greatest GSH cellular concentration because its basolateral membrane has less capacity for cell-to-bath transport of GSH; and (*4*) GSH can be secreted intact from the peritubular compartment into the tubular lumen.

Relatively little is known about the mechanisms involved in the transport of glutathione (GSH) by the intact epithelial cells lining the renal proximal tubule. Interest in characterizing the renal handling and transport of GSH did not arise until about the early 1980s. The impetus for beginning to characterize the transport of GSH in the kidney came mainly from the discovery that GSH is present in the plasma and that the kidneys can extract GSH from the plasma at a rate that is greater than that which can be accounted for by glomerular filtration and subsequent absorption (1,2). Thus, it appeared that GSH and/or its constituent amino acids are likely taken up by renal tubular epithelial cells at both the luminal and basolateral membranes.

It is generally accepted that GSH that is filtered and/or secreted into the lumen of proximal tubular segments is first rapidly broken down into its constituent amino acids (glutamate, cysteine, and glycine) after the sequential actions of the enzymes γ-glutamyltransferase (γ-GT) and cysteinylglycinase. Then, these constituent amino acids are absorbed rapidly and efficiently within the proximal tubular segments via the actions of various amino acid transporters (3).

Despite the large amount of data regarding the luminal processing and reclamation of filtered or secreted GSH, very little is known about whether GSH is, or can be, transported, as an intact tripeptide, across the luminal plasma membrane of proximal tubular epithelial cells in a lumen-to-cell direction. About the only direct evidence for the luminal transport of GSH as an intact molecule comes from the studies of Inoue and Morino (4), who demonstrated that GSH can be transported across isolated brush-border membrane vesicles following the inactivation of γ-GT by acivicin. Kinetic analysis in this study provided evidence for a membrane potential-dependent mechanism involved in the transport of intact GSH in cortical brush-border membranes. It has been concluded, however, that this mechanism for the transport of intact GSH functions only in the secretion of GSH from proximal tubular epithelial cells into the tubular lumen, and not in luminal absorption of intact GSH. More recent evidence from isolated intestinal brush-border membrane vesicles treated with acivicin indicates that there may indeed be transport of intact GSH, in an absorptive direction, mediated by a Na^+-independent system (5,6).

By contrast, there is somewhat more substantial evidence for the transport of GSH, as an intact molecule, into proximal tubular epithelial cells across the basolateral membrane. Studies of the uptake of GSH by basolateral membrane vesicles isolated from the kidney and small intestine of rats tend to

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indicate that GSH is taken up at the basolateral membrane and that the mechanism involved is Na^+-dependent and electrogenic (7–9). This basolateral membrane transport of GSH has also been shown to be independent of the catalytic activity of γ-GT (8–11). In addition, basolateral extracellular-to-cell GSH transport can be inhibited by other γ-glutamyl amino acids, suggesting specificity for the γ-glutamyl moiety (8). This Na^+-dependent transport mechanism for GSH exhibited saturation kinetics, while a separate Na^+-independent uptake mechanism displayed linear uptake of GSH to 10 mM (8,9).

Much less is known about the transport of GSH from the cytoplasm of the proximal tubular epithelial cells into the luminal space (secretion). The amount of GSH excreted in the urine by acivicin-treated rats (to prevent the enzymatic degradation of GSH in the luminal compartment) was 40-fold greater than the filtered load (10). Similar results have been obtained from surface proximal tubules of rats using the *in vivo* microperfusion technique (11). Inoue and Morino (4) showed transport of intact GSH by renal brush-border membrane vesicles in which γ-GT was inactivated by acivicin. Kinetic analysis of the data from this study provided evidence for the presence of a secretory transport mechanism for GSH located in brush-border membrane that was membrane potential-dependent. More recently, we established the presence of luminal secretion of cellularly synthesized GSH in isolated and perfused proximal tubules of the rabbit, quantifying the secretion and synthesis rates of GSH in the S_1, S_2, and S_3 segments (12).

To extend our current knowledge regarding the mechanisms involved in the renal proximal tubular transport of GSH, we designed experiments to quantify the lumen-to-bath and bath-to-lumen transport of GSH in intact proximal tubular cells located in isolated, perfused S_1, S_2, and S_3 segments of the proximal tubule of the rabbit. More specifically, we tested the hypothesis that GSH can be transported, under the appropriate conditions, as an intact tripeptide, into proximal tubular epithelial cells *in situ* across both the luminal and basolateral membranes.

The findings in this present study provide for the first time actual rates of cell-to-lumen (secretion), lumen-to-cell, and cell-to-bath rates of transport of intact GSH across the luminal and basolateral membranes, respectively. This confirms our previous evidence that cellularly synthesized GSH is preferentially secreted into the luminal fluid (12).

Materials and Methods

Materials

Perfusing solutions containing tritiated GSH were prepared fresh for each experiment. All isotopes were purchased from New England Nuclear (Boston, MA). The ^{3}H-GSH had a specific activity of 43.8 Ci/mmol and was stored in an aqueous solution with 10 mM dithiothreitol (DTT) under argon and kept at 5°C. The final concentration of ^{3}H-GSH in the perfusing solution (perfusate) was 4.6 μM. When placed in the bathing solution, the final concentration of ^{3}H-GSH was 2.3 μM. This lower concentration in the bathing solution was due to an effort to economize (high cost and the need for great amounts of ^{3}H-GSH characterize bath-to-lumen experiments). However, this 2.3 μM concentration is adequate to measure rates of intact ^{3}H-GSH transport. The concentrations of DTT in the perfusate and bathing solution were 3 and 1.25 mM, respectively. This presence of DTT in the perfusate and bathing solutions prevented the ^{3}H-GSH from oxidizing during experiments. The ^{3}H label on the GSH was located on the glycinyl residue. ^{14}C-polyethylene glycol (^{14}C-PEG) was used as a volume marker in all experiments. It had a specific activity of 11.0 mCi/g and a concentration of 2.3 mM in the perfusate or bathing solution. With the exception of the anesthetics (described below), all other chemicals were purchased from Sigma Chemical Co. (St. Louis, MO). When ^{3}H-GSH was analyzed with HPLC directly from its container, 94.46 ± 3.42% was associated with the GSH peak, 2.27 ± 0.49% was associated with the glycine peak, and 3.12 ± 0.14% was associated with the GSSG (glutathione disulfide) peak. No other peaks had any ^{3}H above background associated with it.

Solutions

A standard artificial perfusion media (APM) was used for perfusion and bathing solutions that contained (in mM): 145 Na^+, 140 Cl^-, 5.0 K^+, 2.5 Ca^{2+},1.2 Mg^{2+}, 1.2 SO_4^{2-}, 2.0 HPO_4^{2-}-$H_2PO_4^-$, 0.5 L-glutamate, and 1.0 D-glucose. Osmolality was adjusted to 290 mosmol/kg of water and pH to 7.4. The vital dye FD&C Green (250 nM) was added to the perfusion solution only. When acivicin was used, it was added to the perfusion solution to bring the concentration to 1 mM (12). At this concentration, γ-GT was completely inhibited while causing no cellular damage. As determined by HPLC analysis, 2.56 ± 0.76% of the ^{3}H was associated with the glycine peak. No radioactivity above background was associated with the cysteinylglycine peak, and all other ^{3}H was associated with the GSH peak. Because the radioactivity associated with the glycine peak in both instances (directly from the container or from the collectate) was statistically no different, we were confident that γ-GT was completely inhibited.

A sucrose phosphate buffer solution (4°C) was used for tubular dissection. The buffer consisted of 125 mM sucrose, 13.3 mM NaH_2PO_4, and 56 mM Na_2HPO_4. The final pH was 7.4 (adjusted with 1.0 M NaOH) and the osmolality was adjusted to 290 mosmol/kg of water by adding water or sucrose.

Animals

Female New Zealand White, specific pathogen-free rabbits were purchased from Myrtle's Rabbit Farm (Thompson Station, TN). All rabbits were maintained on regular rabbit chow and given water *ad libitum*. Rabbits were anesthetized with ketamine (33 mg/kg body wt) and xylazine (33 mg/kg body wt) purchased from Butler Chemical (Bedford, OH). All experiments were conducted according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Perfusion Methods

The technique of perfusing isolated segments of the nephron used for this study was originally described by Burg *et al.* (13) and subsequently modified in our laboratory (14,15). Data collected for this study were from isolated and perfused S_1, S_2, and S_3 segments of the proximal tubule of the rabbit. The lumen-to-bath transport of ^{3}H-GSH was measured as the disappearance of ^{3}H-GSH from the lumen (J_{DL}, fmol/min per mm), its appearance in the bathing solution (J_{AB}, fmol/min per mm), and its accumulation in the cells of the tubular segments (cell, μM) when ^{3}H-GSH was present in the luminal fluid only. Bath-to-lumen transport of ^{3}H-GSH was measured as the rate ^{3}H-GSH appeared in the luminal fluid (J_{AL}) and accumulation in the tubular cells (cell, μM) when ^{3}H-GSH was present in the bathing

solution only. Individual segments were dissected manually from coronal sections (16–18) and identified as described by Barfuss and Schafer (14) while bathed in the phosphate-sucrose buffer solution.

In lumen-to-bath transport experiments, the perfusate consisted of APM containing 4.6 μM ^{3}H-GSH and 2.3 mM ^{14}C-PEG (polyethylene glycol). ^{14}C-PEG was added to serve as a volume marker.

In bath-to-lumen transport experiments, the bathing solution was APM containing 2.3 μM ^{3}H-GSH and 2.3 mM ^{14}C-PEG. The bathing solution was prepared by combining 50 μl of ^{3}H-GSH stock solution with 50 μl of ^{14}C-PEG stock solution and completely drying them under nitrogen, which evaporated the ethanol and water. The dried ^{3}H-GSH and ^{14}C-PEG were reconstituted into 400 μl of APM and placed into the bathing chamber. Approximately 3 ml of paraffin oil was placed on top of the bathing solution to prevent evaporation of the bathing solution water. The bathing solution was continuously stirred with a reciprocating piston pump.

At the end of each experiment, the perfused tubular segment was harvested with forceps and placed in 10 μl of a 3% TCA solution to extract the cytoplasmic contents, and these contents were analyzed for ^{3}H-GSH, ^{3}H-glycine, ^{3}H-cysteinylglycine, and ^{3}H-glutathione disulfide (GSSG).

Five samples of perfusate, collectate, and bathing fluid samples were collected from each perfused segment. Two were used for HPLC analysis while the remaining three were analyzed for total amount of ^{3}H (Brinkman 5108 scintillation counter; Westbury, NY). The single sample of cellular extract per tubular segment was split in half. One half was used for HPLC analysis and the other half for total ^{3}H analysis.

Confirmation of Steady State

After an individual tubule was perfused and warmed, a period of 15 min was allowed for the attainment of a steady state. Steady-state conditions were confirmed by taking samples from 0 to 20 min after warm-up time and checking for constant values of all measured parameters. Consequently, for each tubule, samples were collected during the 30 min subsequent to the 15-min warm-up period.

Calculations

Mathematical calculations and HPLC analysis for the disappearance, appearance, and cellular concentrations of ^{3}H-GSH and ^{3}H-glycine have been described previously (12).

Statistical Analyses

To determine the rates of transport for each of the three proximal tubular segments, a minimum of five tubules were perfused for each experimental condition. Three or more flux measurements per tubule were made and averaged. The mean values from individual tubules were used to compute an overall mean and SEM for each segment and each experimental condition. Chromatographic samples were analyzed in duplicate. A two-way ANOVA and Tukey honest significant difference *post hoc* test were used to assess differences between means ($P < 0.05$).

Results

Lumen-to-Bath Transport of ^{3}H-GSH

Lumen-to-bath transport studies were done by perfusing tubules with 4.6 μM ^{3}H-GSH (in APM) into the lumen while bathing the tubule in APM. This was done with and without 1.0 mM acivicin in the lumen. Table 1 and Figure 1, A and B, summarize the lumen-to-bath transport of ^{3}H-GSH in the S_1, S_2, and S_3 segments.

In segments perfused without acivicin, the rate of disappearance of ^{3}H-GSH from the lumen (J_{DL}) was 14.38, 14.88, and 14.22 fmol/min per mm for S_1, S_2, and S_3 segments, respectively. In comparison, the J_{DL} for all three segments perfused with 1.0 mM acivicin in the lumen was significantly less, with an average of 1.26 fmol/min per mm for all three segments. Figure 1A shows this marked difference in J_{DL} of ^{3}H-GSH in S_1, S_2, and S_3 segments caused by the presence of 1.0 mM acivicin in the luminal fluid.

The appearance rate of ^{3}H-GSH in the bathing solution during lumen-to-bath transport experiments is presented in Table 1 and Figure 1B. The appearance rate is much less than the corresponding rate of disappearance from the lumen, and there is no marked axial heterogeneity. The presence of acivicin in the lumen significantly reduced the appearance rate of ^{3}H-GSH in the bathing solution.

In experiments in which tubules were perfused without acivicin in the lumen, the cellular concentration of ^{3}H-GSH increased along the length of the proximal tubule from the S_1 (23 μM) and S_2 segment (30 μM) to the S_3 segment (143 μM). The same trend of increasing concentrations of ^{3}H-GSH in the tubular epithelial cells from S_1 to S_3 segments was also seen in the tubules perfused with 1.0 mM acivicin. However, the cellular concentrations of ^{3}H-GSH were significantly less in the tubules perfused with acivicin, ranging from an average

Table 1. Summary of GSH lumen-to-bath transport in the S_1, S_2, and S_3 segments of the rabbit proximal tubule in the absence (−) and in the presence (+) of 1.0 mM acivicin[a]

Segment	GSH Disappearance from Lumen, J_{DL} (fmol/min per mm)	GSH Appearance in Bath, J_{AB} (fmol/min per mm)	GSH Cellular Concentration (μM)	GSH Collectate Concentration (μM)	Perfusion Rate (nl/min)
S_1(−)	14.38 ± 0.69	1.51 ± 0.16	23.07 ± 2.03	0.63 ± 0.09	6.88 ± 0.67
S_1(+)	1.11 ± 0.06	0.62 ± 0.09	4.52 ± 0.78	3.34 ± 0.11	6.31 ± 0.32
S_2(−)	14.88 ± 0.78	3.12 ± 0.79	31.67 ± 11.43	1.02 ± 0.08	8.71 ± 1.13
S_2(+)	0.98 ± 0.10	0.71 ± 0.06	14.01 ± 2.00	2.55 ± 0.06	6.03 ± 0.13
S_3(−)	14.22 ± 1.40	2.09 ± 0.54	143.48 ± 17.94	0.29 ± 0.07	8.15 ± 0.93
S_3(+)	1.71 ± 0.20	0.22 ± 0.03	65.54 ± 4.24	2.44 ± 0.26	7.00 ± 1.27

[a] Perfusion solution contained 4.6 μM ^{3}H-GSH. Data are expressed as mean ± SEM of at least five tubules for each group. These data are companion to data in Table 2 on production of ^{3}H-glycine (same tubules). GSH, glutathione.

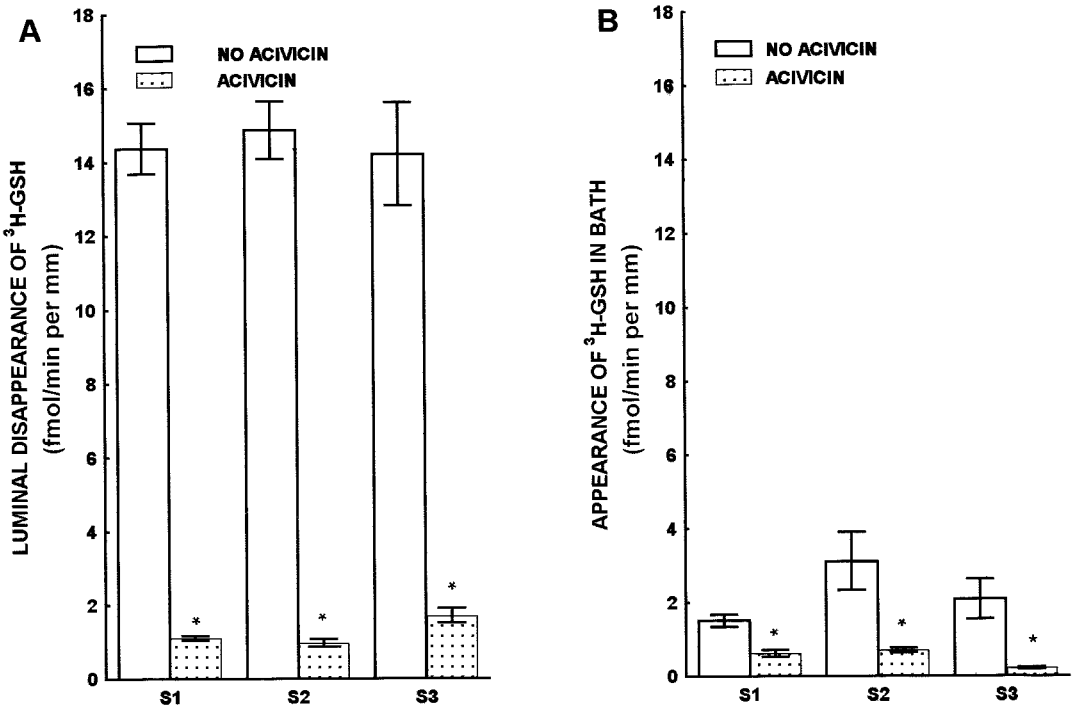

Figure 1. (A) Comparison of ^{3}H-glutathione (^{3}H-GSH) disappearance from the lumen in isolated perfused S_1, S_2, and S_3 segments of the rabbit proximal tubule perfused with 4.6 μM ^{3}H-GSH in artificial perfusion media (APM) in the presence and absence of 1.0 mM acivicin in the lumen. (B) Comparison of ^{3}H-GSH appearance in the bathing solution in S_1, S_2, and S_3 segments perfused with 4.6 μM ^{3}H-GSH in APM in the presence and absence of 1.0 mM acivicin (same tubules as in A). Each bar is the mean of five tubules with standard error lines. *$P < 0.05$.

low of 4.5 μM in the S_1 segments to a high of 65 μM in the S_3 segments.

The collectate concentration of ^{3}H-GSH in the lumen-to-bath experiments was substantially and significantly greater in the tubules perfused with acivicin than in tubules perfused without acivicin. This was the case for all three segments of the proximal tubule.

Table 2 summarizes the data from the lumen-to-bath experiments on the production rate of ^{3}H-glycine from luminal degradation of ^{3}H-GSH in S_1, S_2, and S_3 segments with and without 1.0 mM acivicin present in the luminal compartment. Luminal production rates of ^{3}H-glycine (12 fmol/min per mm) in S_1, S_2, and S_3 tubules perfused without acivicin were not statistically different from each other. However, the production rate of ^{3}H-glycine in the group of tubules perfused with acivicin (0.15 fmol/min per mm) was significantly lower in magnitude than the tubules perfused without acivicin.

There was an increased cellular concentration of ^{3}H-glycine along the proximal tubule ($S_1 \rightarrow S_3$), with or without acivicin in the lumen. Under both conditions, the S_3 segments had the highest cellular concentrations, exhibiting the same trend as the ^{3}H-GSH. But in segments perfused with acivicin, the ^{3}H-

Table 2. Summary of the production rate, rate of appearance in the luminal and bathing solutions, cellular concentration, and collectate concentration of ^{3}H-glycine when the S_1, S_2, and S_3 segments of the rabbit proximal tubule were perfused with 4.6 μM ^{3}H-GSH[a]

Segment	^{3}H-Glycine Production (fmol/min per mm)	^{3}H-Glycine Appearance in Lumen (fmol/min per mm)	^{3}H-Glycine Appearance in Bath (fmol/min per mm)	[^{3}H-Glycine] in Cells (μM)	^{3}H-Glycine Collectate Concentration (μM)	Perfusion Rate (nl/min)
$S_1(-)$	11.78 ± 0.54	6.75 ± 0.21	5.75 ± 0.57	6.24 ± 1.37	1.23 ± 0.10	6.88 ± 0.67
$S_1(+)$	0.17 ± 0.06	0.12 ± 0.04	0.05 ± 0.02	0.29 ± 0.05	0.09 ± 0.03	6.31 ± 0.32
$S_2(-)$	12.13 ± 0.70	5.85 ± 0.26	6.28 ± 0.81	29.15 ± 5.42	1.34 ± 0.21	6.06 ± 0.62
$S_2(+)$	0.16 ± 0.03	0.11 ± 0.02	0.05 ± 0.01	3.32 ± 0.38	0.07 ± 0.01	6.03 ± 0.29
$S_3(-)$	12.66 ± 1.52	8.93 ± 1.33	3.73 ± 0.85	164.94 ± 20.19	1.46 ± 0.13	8.15 ± 0.93
$S_3(+)$	0.12 ± 0.02	0.07 ± 0.01	0.05 ± 0.01	3.48 ± 0.35	0.21 ± 0.07	7.00 ± 1.27

[a]Data are expressed as mean ± SEM; each group was comprised of at least five tubules. The notations (−) and (+) refer to the absence or presence of 1.0 mM acivicin in the luminal fluid. These data are companion to data in Table 1 (same tubules).

glycine concentration was significantly lower than in their corresponding segment perfused without acivicin.

Bath-to-Lumen Transport of ^{3}H-GSH

Bath-to-lumen transport studies of ^{3}H-GSH were done with S_2 segments perfused with APM and bathed in APM containing 2.3 μM ^{3}H-GSH. The appearance of ^{3}H-GSH in the lumen (J_{AL}), cellular, and collectate concentrations, and mean luminal concentrations were measured for ^{3}H-GSH, ^{3}H-glycine, and ^{3}H-cysteinylglycine.

Table 3 summarizes the results of the bath-to-lumen experiments designed to study the transport of ^{3}H-GSH in S_2 segments perfused through the lumen with or without acivicin. As expected, the appearance of ^{3}H-GSH in the lumen (J_{AL}) and concentrations of ^{3}H-GSH in the collectate were significantly greater when acivicin was present in the lumen.

Cellular concentrations of ^{3}H-GSH were approximately 6 and 9 μM in tubules perfused without and with acivicin, respectively. These were not significantly different from each other but these concentrations were significantly lower than the cellular concentrations of ^{3}H-GSH when the segments were perfused with ^{3}H-GSH through the lumen.

The appearance rate of ^{3}H-glycine in the lumen was significantly greater in the tubules perfused without acivicin (1.95 $\pm$ 0.19 fmol/min per mm) compared with tubules perfused with acivicin (0.05 $\pm$ 0.004 fmol/min per mm) (Table 4). This greater rate of ^{3}H-glycine production is reflected in the greater collectate concentration of ^{3}H-glycine (0.30 *versus* 0.01 μM) and the cellular concentration (2.90 *versus* 0.13 μM) of ^{3}H-glycine in tubules perfused without and with acivicin, respectively.

No significant amount of ^{3}H-cysteinylglycine was found in the bathing solution, collectate, or tubular extract in any of the experiments. This was expected due to the high activity of dipeptidases in the proximal lumen.

Discussion

GSH is found in an abundance in various mammalian tissues including the epithelium lining the renal proximal tubule (3). The renal epithelial cells, particularly those lining the proximal tubule, exhibit high concentrations (5 mM) of GSH compared with micromolar concentrations that are present in the lumen and peritubular fluid (3,19). In the kidneys of rats, GSH has a short half-life of approximately 3.5 s extracellularly and 29 min intracellularly (10), which is in accordance with the high activity of extracellular γ-GT localized mainly on the brush-border membrane of proximal tubular epithelial cells. Due to the extracellular location of γ-GT in the brush-border membrane, intracellular GSH is not accessible to this enzyme for degradation (7,10). On the other hand, GSH that is either filtered or secreted into the lumen of the proximal tubule does gain access to this enzyme, and is hydrolyzed readily to its constituent amino acids, glutamate, glycine, and cysteine.

Absorption and Degradation of Luminal ^{3}H-GSH

When tubules were perfused with ^{3}H-GSH and γ-GT was not inhibited, the rates of disappearance of ^{3}H-GSH from the luminal fluid (J_{LD}) of perfused S_1, S_2, and S_3 segments were similar (approximately 14 fmol/min per mm). The majority (approximately 86%) of ^{3}H-GSH that disappeared from the lumen was linked to the activity of γ-GT and cysteinylglycinase, resulting in the degradation of ^{3}H-GSH into ^{3}H-glycine, cysteine, and glutamate. In proximal tubular segments perfused without acivicin, the rates of ^{3}H-glycine production due to the degradation of ^{3}H-GSH were only slightly less than the rates of disappearance of ^{3}H-GSH from the lumen (12 *versus* 14 fmol/min per mm). The difference in these two rates could be due to the absorption of intact ^{3}H-GSH. It is presumed that the constituent amino acids of GSH were absorbed into the tubular epithelial cells for metabolism or transport across the basolateral membrane into the bathing solution. The rates of production of ^{3}H-glycine for all three segments of the proximal tubule were the same at about 12.5 fmol/min per mm when γ-GT was functioning and the rates of delivery of ^{3}H-GSH into the lumen of all proximal tubular segments were about equal (36 fmol/min). From these findings, we conclude that the amount of γ-GT was either evenly distributed along the three segments of the proximal tubule or ^{3}H-GSH was recycling between the luminal and cellular fluid. This ^{3}H-GSH recycling could result from intracellular synthesis of ^{3}H-GSH as a result of high levels of cellular ^{3}H-glycine (Table 2). This apparent lack of longitudinal heterogeneity contradicts previous studies showing that the amount of γ-GT increases along the proximal tubule (convoluted $<$ straight) (20,21). Therefore, the possibility of ^{3}H-GSH recycling seems very likely.

There is evidence for di- and tripeptides being absorbed intact in renal brush-border membrane vesicles and isolated-perfused proximal tubules (22–24), but no clear evidence has been provided to support the hypothesis that intact luminal absorption of GSH can occur in the proximal tubule of the

Table 3. Summary of bath-to-lumen transport of ^{3}H-GSH in the S_2 segment of the rabbit proximal tubule in the absence (−) and in the presence (+) of 1.0 mM acivicin in the luminal perfusion fluid[a]

Segment	GSH Appearance in Lumen, J_{AL} (fmol/min per min)	GSH Cellular Concentration (μM)	GSH Collectate Concentration (μM)	Perfusion Rate (nl/min)
S_2(−)	0.16 $\pm$ 0.03	6.07 $\pm$ 0.49	0.12 $\pm$ 0.003	8.71 $\pm$ 1.13
S_2(+)	3.01 $\pm$ 0.21	9.45 $\pm$ 1.04	0.27 $\pm$ 0.02	5.85 $\pm$ 0.28

[a] Bathing solution contained 2.3 μM ^{3}H-GSH. Data are expressed as mean $\pm$ SEM. These data are companion to data in Table 4 (same tubules).

Table 4. Summary of the production and transport of ^{3}H-glycine in the S_2 segment of the rabbit proximal tubule in the absence (−) and in the presence (+) of 1.0 mM acivicin in the luminal perfusion fluid during bath-to-lumen ^{3}H-GSH transport experiments[a]

Segment	^{3}H-Glycine Appearance in Lumen, J_{AL} (fmol/min per mm)	^{3}H-Glycine Cellular Concentration (μM)	^{3}H-Glycine Collectate Concentration (μM)	Perfusion Rate (nl/min)
S_2(−)	1.95 ± 0.19	2.90 ± 0.25	0.30 ± 0.07	8.71 ± 1.13
S_2(+)	0.05 ± 0.004	0.13 ± 0.01	0.01 ± 0.002	5.85 ± 0.28

[a] Bathing solution contained 2.3 μM ^{3}H-GSH. Data are expressed as mean ± SEM. These data are companion to data in Table 3 (same tubules).

kidney. Although the disappearance of ^{3}H-GSH from the lumen is significantly lower with acivicin present, there is a measurable transport rate from lumen to cell, supporting the idea of a mechanism for intact GSH transport in this direction. Because there was negligible ^{3}H-glycine or ^{3}H-cysteinylglycine in the lumen or cells of these perfused segments, one can assume that the rate of disappearance of ^{3}H-GSH from the lumen cannot be due or related to the sequential degradation of ^{3}H-GSH to its constituent amino acids. In addition, it can be assumed that unlabeled GSH is being synthesized and secreted into the luminal fluid (decreasing the specific activity of luminal ^{3}H-GSH) and absorbed along with the ^{3}H-GSH (12). Therefore, these measured rates for disappearance of intact ^{3}H-GSH from the luminal fluid may be low estimates due to the endogenous cellular unlabeled GSH being secreted into the luminal fluid. It is also possible that nonlabeled intracellular GSH is being exchanged for luminal ^{3}H-GSH as a mechanism for intact lumen-to-cell transport of ^{3}H-GSH across the luminal membrane. Intact GSH transport from lumen to cell is not the primary method of salvaging filtered GSH, but it may be a backup mechanism. Alternatively, this transporter could have another function altogether but also have a low affinity and capacity for GSH.

Additional evidence for the transport of intact tripeptides in the kidney is provided in renal brush-border membrane vesicles (23). In a strain of rats lacking dipeptidyl peptidase IV, uptake of GSH was enhanced by a pH gradient and an inside-negative membrane potential. Uptake was Na^+-independent, unlike the uptake of GSH from the basolateral membrane described by Lash and Jones (8,9), which was found to be Na^+-dependent. In addition, numerous di- and tripeptides appear to inhibit this transport, suggesting that these peptides compete for transport of GSH. The findings obtained with brush-border membrane vesicles provided the first direct evidence for the presence of an electrogenic tripeptide transporter in renal brush-border membranes. On the basis of this evidence, it is possible that this mechanism may be responsible for the luminal uptake of intact GSH as well. But no definitive evidence for absorption of intact GSH into the epithelial cells of the renal proximal tubule has been provided until the present study.

Cell-to-Bath GSH Transport

As expected, the appearance rates of ^{3}H-GSH in the bathing solution (J_{AB}, approximately 2 fmol/min per mm for all segments) in lumen-to-bath experiments were much less than the corresponding rates of the luminal disappearance of ^{3}H-GSH. Comparing these J_{AB} rates to the reported rates for GSH secretion into the lumen (2.8 to 7 fmol/min per mm) (12) supports the notion that GSH transport across the basolateral membrane from cells into peritubular fluid is not the primary route for GSH transport from the epithelial cells lining the rabbit proximal tubule.

It appears there is axial heterogeneity along the proximal tubule for GSH cell-to-bath transport at the basolateral membrane. Because the J_{AB} values of all segments were the same (approximately 2 fmol/min per mm) and the cellular concentrations of GSH were much different, the cell-to-bath transport mechanism at the basolateral membrane must vary in transport-capacity among the various segments. This is particularly apparent in the S_3 segment. A much greater cellular concentration of GSH is required for the same magnitude of cell-to-bath flux to occur than in the other segments. It would appear that the further a segment is from the glomerulus, the less capacity its basolateral membrane has to transport GSH out of the cell into the extracellular fluid. Because the flux appears to be down an electrochemical potential gradient, the cell-to-bath transport is not considered to be active.

^{3}H-GSH Cellular Concentration in Tubules Perfused with ^{3}H-GSH

There was significant heterogeneity in the cellular concentrations of ^{3}H-GSH among the three segments of the proximal tubule perfused with ^{3}H-GSH in the absence of acivicin. As observed previously (12), there was a progressive increase in the cellular concentration of ^{3}H-GSH from the S_1 to the S_3 segments. One possible source for the cellular ^{3}H-GSH in the proximal tubular cells could be from the transport of intact ^{3}H-GSH from the luminal fluid. Another source may be *de novo* synthesis involving absorbed ^{3}H-glycine that accumulated in the cell as a result of luminal degradation of ^{3}H-GSH. If very little of the intracellular ^{3}H-GSH resulted from synthesis, then the greater than luminal concentrations (4.6 μM) of cellular ^{3}H-GSH in all segments indicate an active transport

mechanism for GSH at the luminal membrane. This conclusion is more apparent when evaluating cellular ^{3}H-GSH in tubules perfused with acivicin in the lumen. With acivicin in the luminal fluid, no significant amount of ^{3}H-glycine was available for synthesis of ^{3}H-GSH. This would indicate that luminal ^{3}H-GSH was actively transported as an intact tripeptide into the cell across the luminal membrane up a concentration gradient in the S_2 and S_3 segments only.

^{3}H-GSH Bath-to-Lumen Transepithelial Transport

Transport of GSH and GSH conjugates has been studied extensively in basolateral membrane vesicles from rat proximal tubules (7,25–27). This bath-to-cytoplasm transport process has been characterized as an electrogenic and Na^+-dependent mechanism. Inhibitor studies have also been done to determine that GSH transport at the basolateral membrane was not dependent on the purported activity of basolateral γ-GT (8). It has also been reported that replacing Na^+ with K^+, choline, or NH_4+ ions did not stimulate the basolateral transport of GSH and that GSSG and γ-glutamyl-glutamate share the same Na^+-dependent basolateral transport mechanism (9).

In the S_2 segment, appearance of ^{3}H-GSH in the lumen from the bath was significantly greater in the tubules perfused with acivicin in the lumen than without acivicin (3.0 *versus* 0.16 fmol/min per mm). This is consistent with the notion that *in vivo* activity of luminal γ-GT degrades GSH in the lumen. This indicates that GSH can be sequestered from the bathing fluid and secreted into the lumen of the S_2 segment of the proximal tubule.

The cellular concentration of ^{3}H-GSH in tubules bathed in a solution containing ^{3}H-GSH and perfused with or without acivicin were not significantly different. However, the activity of γ-GT tended to decrease cellular concentrations of ^{3}H-GSH. In both experimental conditions, the cellular concentrations of GSH were greater than the bathing solution concentration of ^{3}H-GSH. This, coupled with a presumed transmembrane electrical potential of -40 to -70 mV, supports the idea that the transport of intact GSH into the cells at the basolateral membrane is an active process (8,26).

We agree with previous studies that during normal physiologic conditions, any GSH filtered at the glomerulus will be removed from the ultrafiltrate as it passes through the lumen of the proximal tubule. This is accomplished primarily by the complete degradation of GSH to glutamate, cysteine, and glycine (by the actions of γ-GT and cysteinylglycinase), and subsequent absorption of the individual amino acids. But it is also possible that a small fraction of filtered GSH can be recovered by being absorbed intact, although under normal physiologic conditions only a small fraction of GSH is recovered as an intact molecule due to the tremendous amount of γ-GT present in the brush-border membrane. In addition, we conclude that intact absorption of GSH from peritubular fluid by mechanisms at the basolateral membrane contributes to the intracellular concentration of GSH and that this GSH can be secreted into the luminal fluid as an intact molecule. Finally, at a perfusate GSH concentration of 4.6 μM, there is no axial heterogeneity of GSH removal from the lumen along the proximal tubule. However, the transport capacity for GSH from cell to peritubular fluid across the basolateral membrane does display axial heterogeneity, decreasing along the length of the proximal tubule ($S_1 \approx S_2 > S_3$).

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