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Renal Cellular Transport of Exogenous Glutathione: Heterogeneity at Physiological and Pharmacological Concentrations

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ABSTRACT. Properties and kinetics of GSH transport into proximal tubular (PT) and distal tubular (DT) cells from rat kidney were determined to validate further the hypothesis that cellular differences in handling of GSH contribute to the greater susceptibility of DT cells to oxidant injury. PT and DT cells were incubated with a broad range of GSH concentrations, encompassing physiologically relevant (0.001 to 0.1 mM) to pharmacological (0.25 to 5 mM) levels of GSH. GSH uptake in PT cells was rapid, exhibiting an overshoot with a maximum at 1-min incubation. GSH uptake in DT cells reached maximal intracellular levels at 2- to 5-min incubations. GSH uptake in PT cells was resolved into two kinetically distinct processes, with K_m values of 41.7 and 540 μM and V_{max} values of 183 and 4885 pmol/min per 10^6 cells. In contrast, GSH uptake in DT cells was best described by one process, with K_m and V_{max} values of 1480 μM and 2094 pmol/min per 10^6 cells, respectively. Rates of GSH synthesis from 1 mM precursor amino acids were approximately 3-fold faster in PT cells, but rates of cysteine accumulation were 3.5-fold faster in DT cells. Accumulation of intracellular GSH in PT cells was 8-fold faster after incubation with 1 mM GSH than after incubation with 1 mM precursor amino acids. At both a physiological (10 μM) and a pharmacological (5 mM) GSH concentration, uptake exhibited marked Na^+ and energy dependence, sensitivity to substrates for the organic anion and dicarboxylate carriers, and sensitivity to various γ-glutamyl amino acids in PT cells only. Na^+-dependent GSH uptake in PT cells was accounted for completely by activity of the organic anion and dicarboxylate carriers. These results indicate that DT cells possess limited capacity to transport GSH and suggest that exogenous GSH may not be effective in protecting other segments of the nephron besides the PT region from oxidants or other agents that alter GSH status. BIOCHEM PHARMACOL **58**;5:897–907, 1999. © 1999 Elsevier Science Inc.

KEY WORDS. glutathione; kidney; proximal tubule; distal tubule; transport; nephron heterogeneity; oxidant injury

GSH is well-established as a regulator of intracellular redox status and as a protective agent against oxidants and reactive electrophiles in several tissues and cell types. The kidneys, unique among the various tissues in their extensive ability to handle GSH, possess high activities of enzymes to synthesize and degrade GSH and membrane transport proteins to mediate uptake of the intact tripeptide into the cell [1, 2]. Because of these activities, the kidneys play a central role in overall GSH homeostasis. The liver is the primary source of plasma GSH, and depending on the blood vessel examined, plasma in rodents normally contains 10–30 μM GSH [3, 4]. Hence, the kidneys are exposed continuously under normal, physiological conditions to micromolar concentrations of GSH. Oral administration of pharmacological doses of GSH can markedly raise plasma GSH levels to as high as 75–100 μM, leading to significant increases in tissue levels of GSH [5, 6]. This suggests that one can expose the kidneys to supraphysiological (perhaps millimolar) concentrations of GSH to alter cellular redox status, thereby protecting renal cells from oxidants and other GSH-depleting agents.

For many years, the conventional view was that renal extraction of plasma GSH was due solely to glomerular filtration, degradation by GGT† (EC 2.3.2.2) and DP activities on the brush-border membranes of PT cells, and uptake of the constituent amino acids into the renal cell [7]. Several studies on renal GSH transport in the isolated, perfused rat kidney in the late 1970s through the mid-1980s [8–14] provided evidence that either endogenous or administered GSH was extracted from the plasma by both a luminal mechanism, as described above, and a basolateral mechanism. The basolateral mechanism was shown subsequently to involve transport of intact GSH coupled to at least two Na^+ ions; this transport is inhibited by organic

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† *Abbreviations*: BSO, L-buthionine-*S*,*R*-sulfoximine; CySH, cysteine; DMS, dimethylsuccinate; DP, dipeptidase; DT, distal tubular; GGT, γ-glutamyltransferase; Glut, L-glutamate; PAH, *p*-aminohippurate; and PT, proximal tubular.

anions such as PAH and probenecid, suggesting a possible role for the organic anion carrier [15, 16]. The intracellular GSH then can function to provide the epithelial cell with GSH to protect it from oxidant injury [17], or it can undergo turnover by efflux into the lumen by membrane potential-sensitive transport across the brush-border membrane, degradation to the constituent amino acids, and reuptake of the amino acids [18, 19].

These GSH transport systems have been described in renal cortical cells and plasma membrane vesicles from renal cortical homogenates. In both cases, the biological material is derived primarily from the PT region of the nephron so that the observed processes are presumed to be occurring primarily in that nephron segment. In a recent study [20], we confirmed the presence of high activities of GSH transport, degradation, and oxidation in suspensions of freshly isolated PT cells that were enriched from suspensions of renal cortical cells. There is considerable heterogeneity among cell types of the nephron in GSH metabolism [21–23]. Although there is only a modest concentration gradient of GSH along the nephron, with a decrease of approximately one-third from proximal to distal segments, marked differences exist in activities of enzymes of the GSH redox cycle. Thus, we found that PT cells possess significantly higher activities of GSSG reductase (EC 1.6.4.2) and GSH peroxidase (EC 1.11.1.9) than do DT cells [23]. These cell-type differences in GSH status appear to have critical toxicological consequences, as the DT cells are markedly more susceptible than PT cells to acute injury from toxic chemicals or pathological conditions that deplete or oxidize GSH, such as oxidants [23], thiol alkylating agents [24], and ATP depletion [25, 26].

The goal of the present study was to determine the capacity, kinetics, energetics, and substrate specificity of GSH transport and the capacity for *de novo* synthesis of GSH in suspensions of renal PT and DT cells. This work was undertaken to further validate the hypothesis that the greater susceptibility of DT cells to oxidant injury is due to their poor ability to utilize GSH for detoxication processes. Cells were exposed to a broad range of GSH concentrations, encompassing both physiological (up to 0.1 mM) and potential pharmacological (0.25 to 5 mM) exposures. PT cells were shown to possess at least two highly specific mechanisms for uptake of GSH. In contrast, only one system, which had minimal energy requirements or known substrate specificity and was of lower activity relative to that in PT cells, was found in DT cells. These findings provide further support for the hypothesis that the susceptibility of DT cells to oxidants and other chemicals or conditions that alter GSH status is due, in part, to its relatively poor ability to use GSH for detoxication reactions.

MATERIALS AND METHODS

Materials

Collagenase (type I), Percoll, BSA (fraction V), BSO, acivicin [L-(αS,5S)-α-amino-3-chloro-4,5-dihydro-5-isoxazoleacetic acid], DMS, PAH, L-γ-glutamyl-L-glutamate, L-γ-glutamyl-L-glutamine, L-γ-glutamyl-L-cysteine, L-γ-glutamylglycine, L-γ-glutamylglycylglycine, L-γ-glutamyl-*p*-nitroanilide, ouabain, nystatin, and antimycin A were purchased from the Sigma Chemical Co. [^{35}S]-L-Cysteine (specific activity, 1300 Ci/mmol) was purchased from Amersham, and [^{35}S]GSH (specific activity, 142 Ci/mmol) was purchased from New England Nuclear. All other chemicals were of the highest purity available and were obtained from commercial sources.

Isolation of Renal PT and DT Cells

Renal cortical cells were isolated from male Fischer 344 rats (200–300 g; Charles River Laboratories) by collagenase perfusion [27]. Animals were housed in the Wayne State University vivarium, were allowed access to food and water *ad lib.*, and were kept in a room on a 12-hr light–dark cycle. Prior to surgery, rats were anesthetized with i.p. injections of sodium pentobarbital (100 mg/kg body weight). PT and DT cells were separated from the cortical cells by Percoll density-gradient centrifugation [28]. Briefly, cortical cells (5 mL, 5–8 × 10^6 cells/mL) were layered on 35 mL of 45% (v/v) isosmotic Percoll solution in 50-mL polycarbonate centrifuge tubes and were centrifuged at 4° for 30 min at 20,000 *g*. PT cells were pooled from the top 10 mL (least dense) of the gradient, and DT cells were pooled from the bottom 10 mL (most dense) of the gradient. Marker enzyme activities and functional assays were used to confirm the identity and purity of the PT and DT cells. Based on these criteria, the PT and DT cell preparations were estimated to have purities of 97 and 88%, respectively [28].

Cells were suspended at concentrations of 2–3 × 10^6 cells/mL in Krebs-Henseleit buffer supplemented with 25 mM HEPES, pH 7.4, 0.2% (w/v) BSA, 2.5 mM $CaCl_2$, 5 mM glucose, and 5 mM glutamine. Cell concentrations were determined in the presence of 0.2% (w/v) trypan blue on a hemacytometer, and viability was estimated by the number of cells that excluded the dye. Greater than 90% of cells excluded trypan blue at the time of isolation, and the cell preparations maintained greater than 80% viability during the time course of the experiments. All incubations for transport and metabolism measurements were performed in 25-mL polyethylene Erlenmeyer flasks at 37° on a Dubnoff metabolic shaking water bath (60 cycles/min).

Processing of Samples for Measurement of Intracellular GSH

Isolated PT and DT cells (2–3 × 10^6 cells/mL) were preincubated for 20 min with 0.25 mM acivicin to inhibit GSH degradation, 2 mM BSO to inhibit GSH synthesis (except in the experiments where GSH synthesis was measured), and other compounds as indicated in a final volume of 3.5 mL. GSH uptake was initiated by the addition of one of various concentrations of unlabeled GSH (1, 5, 10, 20, 50, 100, 250, and 500 μM, and 1.0, 2.5, and

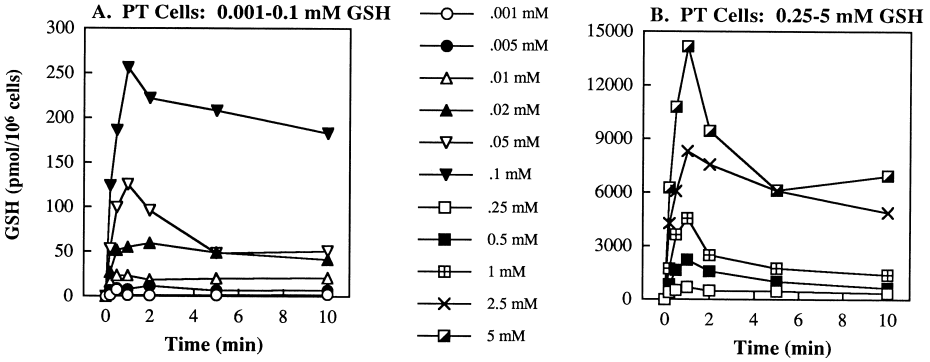

FIG. 1. Time- and concentration-dependent uptake of GSH in renal PT cells. Suspensions of freshly isolated PT cells from rat (2–3 x 10^6 cells/mL) were incubated with from 1 μM to 5 mM GSH containing 4.5 nM [^{35}S]GSH. At the indicated times, aliquots were filtered through 0.45-μm pore size, 25-mm diameter Metricel membrane filters, and radioactivity retained on the filter was counted in a scintillation counter. Results are means of measurements from 3–5 separate cell preparations. SEM values were omitted for clarity, but were generally 5–30% of the mean values.

5.0 mM) and 4.5 nM [^{35}S]GSH. After incubation for 0.2, 0.5, 1, 2, 5, or 10 min, aliquots (0.5 mL) were removed from the incubation mixture and were filtered rapidly through a 0.45-μm pore size, 25-mm diameter Metricel membrane (Gelman Sciences). Then GSH content retained on the filters was measured by one of two methods, scintillation counting or HPLC.

To measure GSH uptake by liquid scintillation counting, filters were rinsed with saline and placed in a vial containing Safety-Solve liquid scintillation fluid (RPI), and the amount of ^{35}S in the retained cells was determined using a Beckman LS 6000IC liquid scintillation counter.

To measure GSH uptake by HPLC, the membrane was washed with 2.0 mL of saline and transferred to a test tube containing 0.5 mL saline and 0.1 mL of 70% (v/v) perchloric acid. Following the addition of bathophenanthroline disulfonate as an antioxidant and an internal standard (L-γ-glutamyl-L-glutamate or L-γ-glutamylglycylglycine), the mixture was vortexed vigorously, centrifuged to remove insoluble material, and a 0.5-mL aliquot was removed for derivatization with iodoacetate and 1-fluoro-2,4-dinitrobenzene for HPLC analysis [20, 29]. Separation of the *S*-carboxymethyl-*N*-dinitrophenyl derivative of GSH was achieved with a reversed-phase, μBondapak amine 10-mm cartridge (8 mm × 10 cm) (Waters Associates) with a Waters model 600E multisolvent delivery system using an ion-exchange method with a methanol–acetate mobile phase and gradient elution. Detection was at 365 nm, and quantitation was done with respect to standards using a Waters model 745B data module. The limit of detection was approximately 50 pmol. To control for variations in sample volumes, peak areas were normalized to the internal standard. L-γ-Glutamyl-L-glutamate was used as the internal standard in all experiments except those in which competition between L-γ-glutamyl-L-glutamate and GSH was assessed. Due to limitations of assay sensitivity, HPLC could be used only to measure GSH uptake with incubating concentrations of ≥0.5 mM GSH.

To verify that the majority of the ^{35}S-label was associated with intact GSH and not degradation products of GSH, the amount of radiolabel in GSH and related thiols or disulfides was determined by HPLC analysis. Aliquots of derivatized samples were injected onto the HPLC, fractions were collected every 2 min, and an aliquot was counted in a Beckman LS 6000IC scintillation counter.

Measurement of GSH Synthesis with [^{35}S]-L-Cysteine

Synthesis of GSH from precursor amino acids was measured by incubating PT or DT cells (2–3 × 10^6 cells/mL) for up to 60 min with 1 mM each of Glut, glycine, and CySH and 4.5 nM [^{35}S]-L-cysteine in a total volume of 3.5 mL. At the indicated times, 0.5-mL aliquots were removed and were derivatized for analysis of GSH and related thiols and disulfides as described above. Cells were first preincubated for 15 min with 0.25 mM acivicin to inhibit GSH degradation.

Data Analysis

All values are means ± SEM, unless otherwise indicated, of measurements from the indicated number of separate cell preparations. Significant differences between means were assessed first by a one-way analysis of variance. When significant "F-values" were obtained with the analysis of variance, the Fisher's protected least significant difference *t*-test was performed to determine which means were significantly different from one another, with two-tail probabilities of less than 0.05 considered significant.

RESULTS

Time and Concentration Dependence of GSH Uptake

Renal PT and DT cells were incubated with a 5000-fold range of GSH concentrations to assess the time and concentration dependence of GSH uptake at both physio-

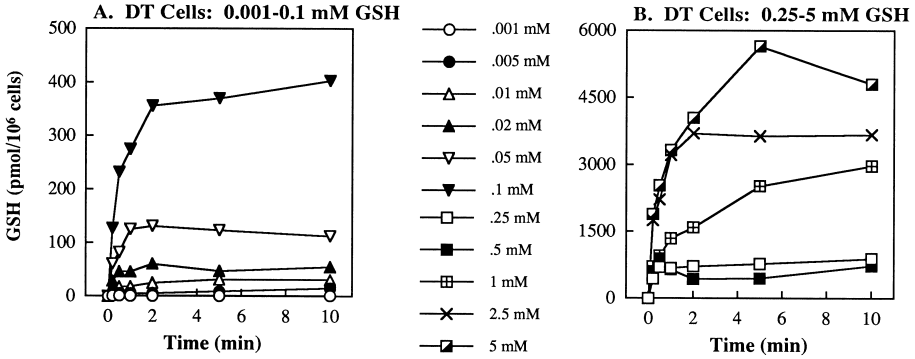

FIG. 2. Time- and concentration-dependent uptake of GSH in renal DT cells. Suspensions of freshly isolated DT cells from rat (2–3 × 10^6 cells/mL) were incubated with from 1 μM to 5 mM GSH containing 4.5 nM [^{35}S]GSH. At the indicated times, aliquots were processed and analyzed as described in the legend to Fig. 1. Results are means of measurements from 3–5 separate cell preparations. SEM values were omitted for clarity, but were generally 5–30% of the mean values.

logical and pharmacological GSH exposure levels (Figs. 1 and 2). Both cell populations exhibited time- and concentration-dependent increases in cellular GSH content. At both low and high ranges of extracellular GSH concentration, the time course of GSH uptake in PT cells exhibited an overshoot, reaching a maximum after 1 min of incubation, after which the cellular content of GSH decreased by from 20% to as much as 50% over the course of the 10-min incubation (Fig. 1). In contrast, the time courses for GSH uptake in DT cells reached maximal levels after approximately 2 min of incubation but did not decrease thereafter (Fig. 2). The maximal intracellular content of GSH achieved with incubating GSH concentrations of 0.001 to 0.1 mM generally was comparable in the two cell types. At incubating GSH concentrations of 0.25 mM and higher, however, PT cells exhibited markedly higher intracellular GSH contents than did DT cells.

Analysis of initial rates of GSH uptake revealed additional differences between the two cell populations. The plot of V versus S for PT cells was fitted to a second-order polynomial and exhibited saturation at GSH concentrations of 2.5 mM and above (Fig. 3A). Analysis of transport kinetics by an Eadie-Hofstee plot showed that uptake in PT cells was described best by a two-component model, using values for substrate concentrations of 0.001 to 0.05 mM for the high-affinity, low-capacity component and 0.1 to 5 mM for the low-affinity, high-capacity component (Fig. 3B).

The plot of V versus S for DT cells was similarly fitted to a second-order polynomial and exhibited saturation at GSH concentrations of 2.5 mM and above (Fig. 4A). Analysis of transport kinetics by an Eadie-Hofstee plot showed that uptake in DT cells was described best by a one-component model (Fig. 4B).

The kinetic parameters obtained from the Eadie-Hofstee plots are summarized in Table 1. Thus, not only is overall capacity for GSH uptake much greater in PT cells, but the involvement of specific carrier proteins is fundamentally different in the two cell types.

The correspondence of radiolabel predominantly with GSH rather than with CySH for both cell types is demonstrated in Fig. 5. A small increase in [^{35}S]CySH was observed only in the PT cells, which is consistent with the high activity of GGT found there and the low activity of GGT found in DT cells. Thus, although GGT activity was inhibited with 0.25 mM acivicin by approximately 98% [20], there is enough residual activity to cause measurable degradation of GSH. In contrast, the residual GGT activity in the DT cells apparently was insufficient to produce any significant degradation of GSH. This point may also ex-

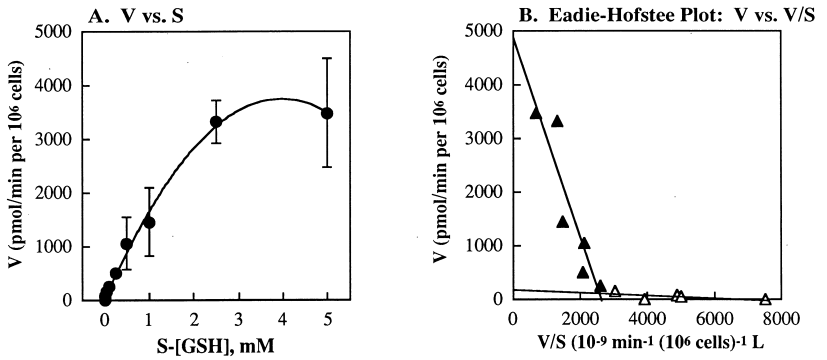

FIG. 3. Kinetics of GSH uptake in renal PT cells. (A) Initial rates of uptake were obtained from time–course data shown in Fig. 1 and are plotted against GSH concentration. Results are means ± SEM of measurements from 3–5 separate cell preparations. The line was drawn by performing a second-order polynomial curve fit: $y = -237.530x^2 + 1880.629x + 43.205$, $r^2 = 0.995$. (B) Eadie-Hofstee plots were constructed by using a two-component model and taking a low and a high range of GSH concentrations and performing linear curve-fitting: 0.001 to 0.05 mM GSH (open triangles): $y = -0.024x + 182.955$, $r^2 = 0.459$; 0.1 to 5 mM GSH (filled triangles): $y = -1.850x + 4885.284$, $r^2 = 0.829$.

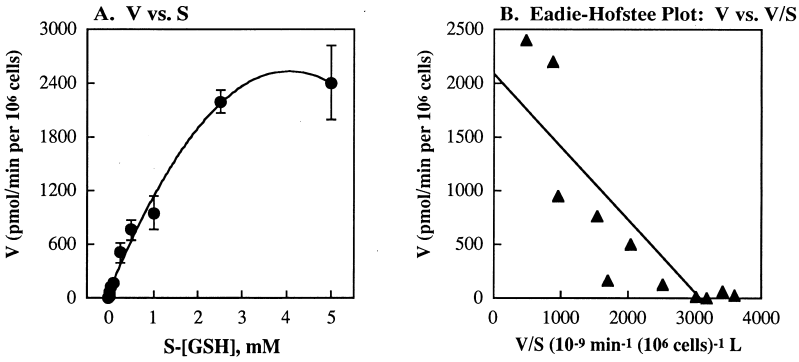

FIG. 4. Kinetics of GSH uptake in renal DT cells. (A) Initial rates of uptake were obtained from time–course data shown in Fig. 2 and are plotted against GSH concentration. Results are means ± SEM of measurements from 3–5 separate cell preparations. The line was drawn by performing a second-order polynomial curve fit: $y = -150.162x^2 + 1221.552x + 57.911$, $r^2 = 0.989$. (B) Eadie-Hofstee plots were constructed by using a one-component model over the entire range of GSH concentrations (0.001 to 5 mM) and performing linear curve-fitting: $y = -0.674x + 2094.347$, $r^2 = 0.718$.

plain the overshoot phenomenon in the time course in PT cells but not in DT cells (cf. Figs. 1 and 2).

Synthesis of GSH from Precursor Amino Acids

The other means to supplement intracellular GSH content is to incubate cells with precursor amino acids. Accordingly, both PT and DT cells were incubated for up to 60 min with 1 mM each of Glut, glycine, and CySH with tracer [^{35}S]CySH, and intracellular contents of GSH and CySH were measured by HPLC and scintillation counting (Fig. 6). From the time–course plots of intracellular GSH accumulation, initial rates of GSH synthesis were calculated to be 0.182 and 0.064 nmol/min per 10^6 cells in PT and DT cells, respectively (Fig. 6A). Thus, PT cells exhibited a 2.8-fold greater capacity to synthesize GSH from precursor amino acids.

In contrast to the pattern of intracellular accumulation of GSH, DT cells accumulated approximately 2.5-fold higher equilibrium levels of CySH than did PT cells (Fig. 6B). Initial rates of CySH accumulation were 0.544 and 1.88 nmol/min per 10^6 cells in PT and DT cells, respectively. Thus, DT cells exhibited a 3.5-fold greater capacity to accumulate CySH from the extracellular medium when GSH synthesis was allowed to occur. Net accumulation of CySH was 2.99- and 29.4-fold greater than accumulation of GSH in PT and DT cells, respectively, illustrating how differently the two cell types regulate thiol status.

TABLE 1. Kinetics of GSH uptake in isolated renal PT and DT cells

Cell type	K_m (μM)	V_{max} (pmol/min per 10^6 cells)
Proximal tubular		
High-affinity, low-capacity	41.7	183
Low-affinity, high-capacity	540	4885
Distal tubular		
Low-affinity, high-capacity	1480	2094

Kinetic parameters are derived from the Eadie-Hofstee plots shown in Figs. 3 and 4, which represent means ± SEM of measurements from 4–5 separate experiments.

Energetics and Substrate Specificity of GSH Uptake

As previous work in isolated membrane vesicles showed that basolateral uptake of GSH is coupled to Na^+, requires metabolic energy, and is partially inhibited by probenecid and PAH [15, 16], we investigated the Na^+ dependence and effects of substrates for the organic anion and dicarboxylate carriers on GSH transport rates in PT and DT cells using both a physiological incubating concentration of GSH (i.e. 10 μM) (Table 2) and a pharmacological incubating concentration of GSH (i.e. 5 mM) (Table 3). Additionally, GSH *S*-conjugates and various γ-glutamyl amino acids are transported across the basolateral membrane by at least one of the systems that also transports GSH [16, 30]. Hence, effects of γ-glutamyl amino acids and the constituent amino acids were tested.

At an incubating GSH concentration of 10 μM, the initial rate of GSH uptake in PT cells was clearly Na^+ dependent and was inhibited by PAH, Glut, and L-γ-glutamylglycine (Table 2). In contrast, no Na^+ dependence was observed for GSH uptake in DT cells with an incubating GSH concentration of 10 μM, but L-γ-glutamylglycine was a potent inhibitor.

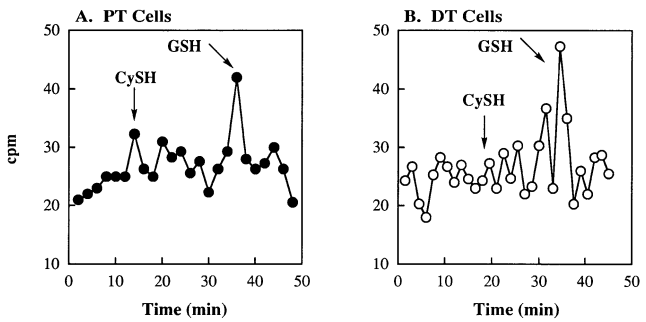

FIG. 5. Correlation between recovery of ^{35}S-label and HPLC analysis of GSH uptake. Aliquots of incubation mixtures from cells incubated with 5 mM GSH and tracer [^{35}S]GSH for 10 min were processed for HPLC analysis as described in Materials and Methods. Fractions coming off the HPLC column were collected and mixed with scintillation fluid, and the quantity of radiolabel in each fraction was determined. The results represent radiolabel tracings from a representative sample from three experiments.

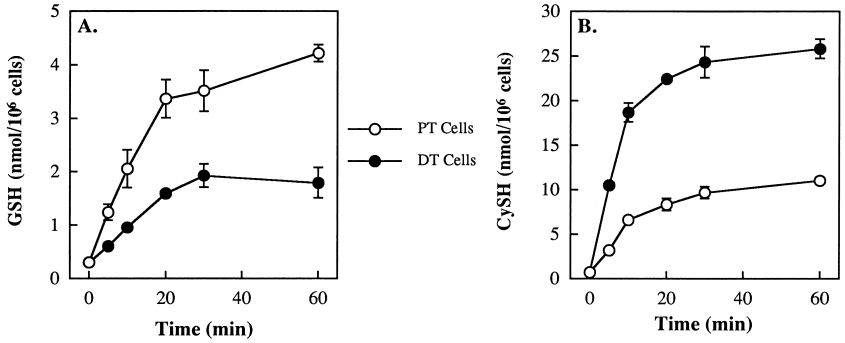

FIG. 6. GSH synthesis and cysteine uptake in incubations of isolated renal PT and DT cells with [^{35}S]-L-cysteine. Isolated PT and DT cells were incubated with 1 mM each of L-cysteine, L-glutamate, and glycine with 4.5 nM [^{35}S]-L-cysteine. At the indicated times, aliquots were removed and filtered, and the counts retained on the filters were determined by scintillation counting. Results are means ± SEM of measurements from three separate cell preparations. Both GSH content (A) and CySH content (B) at all time points except 0 min were significantly different ($P < 0.05$) from one another in the two cell types at corresponding time points.

A more complete study of substrate specificity and energetics was conducted at an incubating GSH concentration of 5 mM (Table 3). Six different γ-glutamyl amino acids were tested to determine if charge or polarity influences the ability to compete with GSH for transport. Similar to the results obtained at the physiological GSH concentration, L-γ-glutamylglycine was a potent inhibitor in PT cells at the pharmacological incubating GSH concentration, decreasing the initial rate of GSH uptake by nearly 60%. L-γ-Glutamyl-L-leucine and L-γ-glutamyl-L-phenylalanine, both of which contain nonpolar, uncharged amino acid moieties, were also efficient inhibitors of GSH uptake. L-γ-Glutamyl-L-histidine, whose amino acid residue should be predominantly uncharged at neutral pH, did not inhibit GSH uptake significantly, although a high degree of variability is likely responsible for the lack of statistical significance. Surprisingly, L-γ-glutamyl-L-cysteine had no significant effect on PT cell GSH uptake. In DT cells, the only significant effect of γ-glutamyl amino acids was a significant increase in GSH uptake in cells incubated with L-γ-glutamyl-L-cysteine or L-γ-glutamyl-L-glutamine. The effect was particularly large with the former compound, which nearly doubled the initial rate of GSH uptake. Of the constituent amino acids, only Glut in PT cells caused a statistically significant decrease in GSH uptake.

The most dramatic difference between PT and DT cells was observed in assessments of the energetics of GSH uptake. GSH uptake in PT cells clearly was dependent on Na^+ and metabolic energy, as preincubation of cells with nystatin, antimycin A, or ouabain or incubation under nominally Na^+-free conditions produced 29–56% inhibition. In marked contrast to this, the metabolic inhibitors or the absence of Na^+ had no effect on GSH uptake in DT cells, indicating that any transport occurs by a passive, facilitated mechanism.

DMS and PAH, which are substrates for the dicarboxylate and organic anion transporters, respectively, both significantly inhibited GSH uptake in PT cells but had no effect in DT cells, which agrees with the known cell-type distribution of these carriers.

To investigate further the relative roles of the dicarboxylate and organic anion transporters, cells were incubated with 5 mM GSH and several combinations of inhibitors (each at 20 mM), both in the presence and absence of

TABLE 2. Energetics and substrate specificity of uptake of 10 μM GSH in renal PT and DT cells

	PT cells		DT cells	
Incubation	GSH uptake (pmol/min per 10^6 cells)	% of Control	GSH uptake (pmol/min per 10^6 cells)	% of Control
Buffer	50.3 ± 4.0	100	24.8 ± 1.3	100
γ-Glu-Gly	38.2 ± 5.4*	75.9	13.4 ± 5.4*	54.0
Cysteine	41.5 ± 6.7	82.5	25.5 ± 4.0	103
Glutamate	32.8 ± 6.0*	65.2	25.5 ± 4.0	103
Glycine	39.5 ± 6.0	78.5	18.8 ± 4.0	75.8
Na^+-free	20.1 ± 2.0*	40.0	25.5 ± 4.0	103
PAH	30.2 ± 3.4*	60.0	23.5 ± 2.7	94.8

Isolated cells (2–3 × 10^6/mL) were incubated for 0.2, 0.5, 1, 2, 5, and 10 min with 10 μM GSH containing [^{35}S]GSH and either buffer or 40 μM of the indicated compound. To assess Na^+-dependence, choline chloride replaced NaCl in the Krebs-Henseleit buffer. Results are expressed as rates of GSH uptake and are means ± SEM of measurements from four separate cell preparations.

*Significantly different ($P < 0.05$) from the corresponding control incubations.

TABLE 3. Energetics and substrate specificity of uptake of 5 mM GSH in renal PT and DT cells

Incubation	PT cells		DT cells	
	GSH uptake (nmol/min per 10^6 cells)	% of Control	GSH uptake (nmol/min per 10^6 cells)	% of Control
γ-Glutamyl compounds				
Buffer	4.97 ± 0.30	100	2.63 ± 0.20	100
γ-Glu-Cys	4.13 ± 0.67	83.1	5.10 ± 0.65*	194
γ-Glu-Gln	3.38 ± 0.55*	68.0	3.53 ± 0.37*	134
γ-Glu-Gly	2.13 ± 0.39*	42.9	2.22 ± 0.27	84.4
γ-Glu-His	3.28 ± 1.10	66.0	2.85 ± 0.57	108
γ-Glu-Leu	2.90 ± 0.42*	58.4	2.83 ± 0.40	108
γ-Glu-Phe	2.73 ± 0.05*	54.9	2.90 ± 0.35	110
Constituent amino acids				
Buffer	4.33 ± 0.67	100	2.18 ± 0.32	100
Glutamate	2.72 ± 0.31*	62.8	2.37 ± 0.52	109
Cysteine	3.33 ± 0.65	76.9	3.00 ± 0.62	138
Glycine	4.13 ± 1.01	95.4	2.80 ± 0.35	128
Energetics and anion transport inhibitor				
Buffer	4.70 ± 0.28	100	2.50 ± 0.42	100
Nystatin	3.08 ± 0.47*	65.5	2.55 ± 0.38	102
Antimycin A	3.35 ± 0.45*	71.3	2.28 ± 0.45	91.2
Ouabain	2.05 ± 0.28*	43.6	2.00 ± 0.45	80.0
Na^+-free	2.82 ± 0.47*	60.0	1.97 ± 0.43	78.8
DMS	2.25 ± 0.33*	47.9	2.83 ± 0.27	113
PAH	2.15 ± 0.14*	45.7	2.71 ± 0.30	108

Isolated cells (2–3 × 10^6/mL) were incubated for 0.2, 0.5, 1, 2, 5, and 10 min with 5 mM GSH containing [^{35}S]GSH and either buffer or 20 mM of the indicated compound, except for nystatin, antimycin A, and ouabain, which were present at concentrations of 0.25 mg/mL, 1 μM, and 0.1 mM, respectively. To assess Na^+-dependence, choline chloride replaced NaCl in the Krebs-Henseleit buffer. Results are expressed as rates of GSH uptake and are means ± SEM of measurements from four separate cell preparations. Abbreviations: γ-Glu-Cys, L-γ-glutamyl-L-cysteine; γ-Glu-Gln, L-γ-glutamyl-L-glutamine; γ-Glu-Gly, L-γ-glutamylglycine; γ-Glu-His, L-γ-glutamyl-L-histidine; γ-Glu-Leu, L-γ-glutamyl-L-leucine; γ-Glu-Phe, L-γ-glutamyl-L-phenylalanine; DMS, dimethylsuccinate; and PAH, *p*-aminohippurate.

*Significantly different ($P < 0.05$) from the corresponding control incubations.

sodium ions, and initial rates of uptake of 5 mM GSH were measured in PT cells (Fig. 7A) and DT cells (Fig. 7B). As shown above, both PAH and DMS significantly inhibited uptake of GSH in normal, Na^+-containing buffer in PT cells but not in DT cells. When rates of GSH uptake were also measured in the absence of Na^+ and Na^+-dependent rates were calculated, both PAH and DMS were found to inhibit GSH uptake by nearly 70% in PT cells. Combination of the two inhibitors caused additional inhibition of total GSH uptake (i.e. Na^+-dependent + Na^+-independent) and approximately 95% inhibition of the Na^+-dependent GSH uptake in PT cells. Total uptake of 5 mM GSH in DT cells was only 60% of that in PT cells, while the Na^+-dependent rate in DT cells was only 23% of that in PT cells.

γ-Glu-Gly was chosen as a test γ-glutamyl amino acid, as it was one of the most potent competitive inhibitors of GSH uptake (cf. Tables 2 and 3). γ-Glu-Gly inhibited total, Na^+-independent, and Na^+-dependent GSH uptake by slightly more than 50% in PT cells but had no significant effect in DT cells (Fig. 7). Combination of γ-Glu-Gly, PAH, and DMS in incubations of PT cells, rather than producing complete inhibition of GSH uptake, produced similar inhibition of total, slightly more inhibition of Na^+-independent, and less inhibition of Na^+-dependent uptake than the combination of PAH and DMS alone.

DISCUSSION

The biochemical and functional heterogeneity of the mammalian nephron, as noted by differences in the activities of transporters and various drug metabolism enzymes, helps determine the susceptibility of the various cell types to injury from toxic chemicals or pathological conditions. We previously used an enriched preparation of freshly isolated PT and DT cells from rat kidney as cellular models to explore nephron heterogeneity [28], and have focused primarily on differences in cellular handling of GSH as an important determinant of susceptibility. This focus was based on two factors. First, GSH functions as a nucleophile and as a reductant in numerous reactions and is thus a critical element in cellular defense against toxic electrophiles or oxidant stress. Second, the kidneys are the major sites for the processing of GSH in the body, and some differences in nephron segments in the specific mechanisms by which this processing occurs are known [1, 2]. The pathways of GSH metabolism and transport were characterized recently in freshly isolated PT cells from the rat kidney [20]. In that study, relatively high concentrations of GSH (0.5 to 5 mM) were used to quantify cellular uptake, oxidation, and degradation of GSH. In earlier studies [23–26], we observed that DT cells were markedly more sensitive than PT cells to cellular injury from a variety of chemicals or conditions that deplete or oxidize GSH.

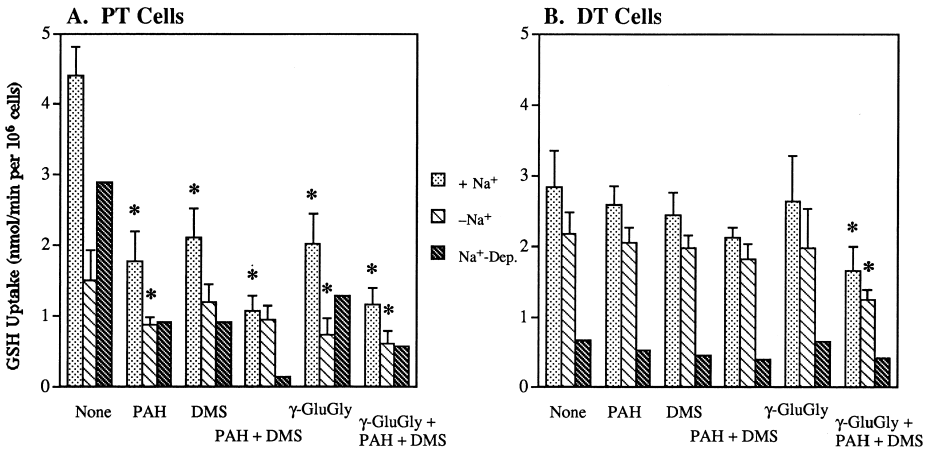

FIG. 7. Role of organic anion carriers in the uptake of GSH by renal PT and DT cells. Isolated cells (2–3 × 10^6/mL) were incubated for 0.2, 0.5, 1, 2, 5, and 10 min with 5 mM GSH and either buffer containing sucrose or 20 mM each of the indicated compound Osmolarity was kept constant by adjusting the concentration of sucrose in the incubation buffer. To assess Na^+ dependence, choline chloride replaced NaCl in the Krebs-Henseleit buffer. Results are expressed as initial rates of GSH uptake and are means ± SEM of measurements in the presence or absence of Na^+ from 3–7 separate cell preparations. Because transport in the presence and absence of Na^+ was measured in separate cell preparations due to limitations in amounts of cells, Na^+-dependent rates were calculated by subtracting the means for uptake in the absence of Na^+ from those for uptake in the presence of Na^+ and could not be calculated from individual measurements. Key:(*) significantly different ($P < 0.05$) from the corresponding control incubations (i.e. no addition). Abbreviations: γ-Glu-Gly, L-γ-glutamyl-glycine; PAH, p-aminohippurate; and DMS, dimethylsuccinate.

Although we [23] and others [21, 22] showed that activities of certain critical, GSH-dependent enzymes were higher in PT cells than in more distal segments of the nephron, the ability to transport GSH was not compared in these cells or nephron segments.

In the present study, we examined the characteristics of GSH uptake in PT and DT cells with both physiologically relevant (0.001 to 0.1 mM) and higher, pharmacological (0.25 to 5 mM) extracellular concentrations of GSH. Based on previous data on activities of GSH-dependent drug metabolism enzymes, we hypothesized that DT cells would be less active than PT cells in GSH transport and that transport would exhibit different kinetics and specificity. The objective of incubating cells with "physiological" and "pharmacological" concentrations of GSH was 2-fold, to simulate the concentrations of GSH to which the kidneys normally are exposed and to incubate renal cells with high concentrations of GSH that can be used to modulate cellular GSH status and potentially protect the cells from various forms of chemically induced or pathological injury.

Comment is necessary on the appropriateness of the use of freshly isolated renal cells as models for the study of plasma membrane transport. One hallmark of renal metabolite transport is the polarity of the brush-border and basolateral plasma membranes with respect to morphology and localization of enzymes and transport proteins. In fact, this polarity enables such processes as secretion and reabsorption, which are directional in nature, to occur with high efficiency. A potential problem with the isolated cell model is that there is no physical separation between the brush-border and basolateral membranes, i.e. all membrane surfaces are equally exposed to the incubating medium. Hence, it can be difficult to distinguish between processes that are occurring on one or the other membrane surface. Renal tubular secretion of either exogenous GSH [31, 32] or GSH synthesized from precursor amino acids within PT cells [33] has been demonstrated, indicating that directional flux of GSH occurs in the intact kidney.

Our primary interest in the use of these isolated cells as biological models has been to investigate factors that contribute to susceptibility to chemical and pathological injury [34]. For this purpose, the isolated cells have several advantages and are quite useful. Hence, although membrane polarity is not maintained, thus potentially making it difficult to identify the site of transport, the cells can be used to assess the overall role of transport and cellular accumulation processes in susceptibility to injury. With respect to the transport of GSH and related compounds, comparison of some of the data obtained previously in isolated renal cells [17, 20, 30, 35] with those obtained with purified basolateral and brush-border plasma membrane vesicles [15, 16, 19] suggests that the transport properties observed in the isolated cells predominantly reflect processes that are occurring on the basolateral membrane.

Observations in the present study on the rates, kinetics, substrate specificity, and energetics of GSH uptake in renal PT and DT cells suggested that GSH transport across the cellular plasma membranes in PT cells is mediated by specific, energy-dependent carriers, whereas that in DT cells likely occurs by facilitated diffusion. Kinetic analysis of GSH uptake in PT cells was consistent with the presence of at least two transporters, a high-affinity, low-capacity carrier and a low-affinity, high-capacity carrier. Under physiological conditions where the renal PT cells are exposed to micromolar concentrations of GSH, most of the transport of GSH will be mediated by the high-affinity, low-capacity

system. In contrast, under pharmacological conditions in which one would expose the PT cells to relatively high (i.e. millimolar) concentrations of GSH for the goal of increasing cellular GSH content and protecting the cells from injury associated with GSH depletion or oxidation, the majority of the transport of GSH will be mediated by the low-affinity, high-capacity system.

Comparison of amounts of accumulation of intracellular GSH by incubating PT cells with extracellular GSH or with precursor amino acids indicates that exposure of cells to intact GSH produces a more rapid increase in intracellular GSH content (1.47 nmol GSH/min per 10^6 cells for uptake of 1 mM GSH vs 0.18 nmol GSH/min per 10^6 cells for uptake of 1 mM each of CySH, Glut, and glycine and synthesis of GSH). After 10 min of incubation with either GSH or the amino acids, however, intracellular contents of GSH were comparable. These findings agree with previous *in vivo* studies [5, 6] that showed that oral administration of a bolus of GSH produced a much greater increase in tissue GSH contents than oral administration of a bolus of the constituent amino acids that comprise GSH.

De novo synthesis and intracellular accumulation of GSH from precursor amino acids were approximately 3-fold faster in PT cells. However, cellular accumulation of CySH was 3.5-fold greater in DT cells. This may be due to either more rapid uptake of the amino acids in DT cells, a greater use of CySH for synthesis of GSH or proteins in PT cells, or a combination of the two factors. Whatever the mechanism is for this difference, it is clear that thiol metabolism and homeostasis differ in the two cell types. Previous measurements of GSH synthesis in isolated nephron segments or tubules [21, 22] and in isolated renal PT and DT cells [23, 36, 37] showed only modestly higher rates in the PT region than in more distal segments of the nephron. In our previous studies with these cell preparations [23, 36, 37], an enzyme-linked assay was used to measure GSH synthesis, whereas in the present study direct measurement of GSH synthesis from precursor amino acids was used. In any case, the PT region clearly has a greater capacity to obtain GSH by synthesis from precursors or by transport from the extracellular space.

Examination of energy dependence and substrate specificity in PT cells at both 10 μM and 5 mM extracellular GSH concentrations showed a marked dependence on Na^+ and metabolic energy, interaction with γ-glutamyl amino acids, and interaction with organic anions and dicarboxylates. These properties agree well with those previously described for GSH uptake in isolated basolateral membrane vesicles [15, 16]. To help discern which carriers might be involved in GSH uptake into renal PT cells, combinations of inhibitors were tested in both the presence and absence of Na^+ in the incubation medium (cf. Fig. 7). These findings need to be considered in the context of the known organic anion carriers that have been identified on renal PT cell brush-border and basolateral plasma membranes [see Refs. 38–40 for recent reviews]. The basolateral membrane contains a PAH/2-oxoglutarate exchanger that is the primary system involved in the first transport step of secretion of organic anions. This exchanger is not directly dependent on membrane potential, but an effect of an inwardly directed Na^+ gradient can be demonstrated, suggesting some interaction or linkage with other carriers. The basolateral membrane also contains a Na^+/dicarboxylate cotransporter that is electrogenic, transporting three Na^+ ions with each dicarboxylate molecule, and the ATP driven, multidrug related transport protein (MRP). MRP normally transports organic anion conjugates into the interstitium, so it may only play a role in reabsorption and not in uptake. The brush-border membrane contains an anion/anion exchanger, where the anion can be PAH, urate, Cl^-, OH^-, HCO_3^- or others. A Na^+/dicarboxylate exchanger (NaDC-1) and a recently cloned organic anion/anion exchanger, oatp-1, are also present on the luminal membrane and potentially may function in GSH uptake in PT cells. Further work is needed to directly establish a role for any of these carriers in GSH transport in renal PT cells. However, some conclusions can be reached based on the inhibitor studies mentioned above.

The nearly complete inhibition of Na^+-dependent GSH uptake in PT cells by the combination of PAH and DMS (cf. Fig. 7A) indicates that the basolateral PAH/2-oxoglutarate carrier and the Na^+/dicarboxylate transporter likely account for all of the observed Na^+-dependent transport of GSH in these cells. PAH also significantly inhibited Na^+-independent GSH uptake, suggesting that the brush-border membrane anion/anion exchanger oatp-1 may account for transport as well. The potent inhibition of GSH uptake by γ-Glu-Gly and the lack of complete inhibition of GSH uptake by the combination of γ-Glu-Gly and PAH + DMS suggests that γ-Glu-Gly is competing for GSH for one or more of these carriers and that it may exchange for GSH, thereby increasing the measured GSH uptake as compared with that in the presence of PAH + DMS alone. γ-Glu-Gly transport into PT cells was extremely rapid and occurred at a faster rate than that of GSH (data not shown), which is consistent with the proposed exchange with GSH, leading to enhanced GSH uptake.

The specificity studies in DT cells contrasted sharply with those in PT cells, which might be expected inasmuch as the various organic anion carriers described above are only present in the proximal tubules. Little dependence on Na^+ or metabolic energy was observed, although the kinetics of uptake and accumulation of GSH did exhibit saturability. It seems likely, therefore, that any GSH uptake that occurs in DT cells is due to facilitated diffusion. Such a carrier would likely have little physiological role because most of the GSH in the renal plasma or lumen would be extracted by the PT cells. Under toxic or pathologic conditions, where selective damage to PT cells might occur, increased delivery of GSH to more distal segments of the nephron would likely occur, thereby providing substrate to any carrier(s) in the DT cells. We recently found that the transport of GSH from cytosol into renal cortical mitochondria, which is catalyzed by the dicarboxylate and

2-oxoglutarate carriers of the mitochondrial inner membrane [41], occurs primarily in mitochondria from the PT region of the nephron and that mitochondria isolated from DT cells exhibit minimal GSH transport activity [42].

In summary, this study has provided additional evidence that renal DT cells have a limited capacity to transport and utilize exogenous GSH to maintain cellular redox status. This sharp contrast with the handling of GSH observed in renal PT cells helps explain the greater susceptibility of DT cells to injury from agents that deplete or oxidize cellular GSH.

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References

1. Lash LH, Glutathione and other antioxidant defense mechanisms. In: *Comprehensive Toxicology* (Ed. Goldstein RS), Vol. 7, pp. 403–428. Elsevier, Oxford, 1997.
2. Lash LH, Jones DP and Anders MW, Glutathione homeostasis and glutathione *S*-conjugate toxicity in the kidney. *Rev Biochem Toxicol* **9:** 29–67, 1988.
3. Anderson ME and Meister A, Dynamic state of glutathione in blood plasma. *J Biol Chem* **255:** 9530–9533, 1980.
4. Lash LH and Jones DP, Distribution of oxidized and reduced forms of glutathione and cyst(e)ine in rat plasma. *Arch Biochem Biophys* **240:** 583–592, 1985.
5. Hagen TM, Wierzbicka GT, Sillau AH, Bowman BB and Jones DP, Bioavailability of dietary glutathione: Effect on plasma concentration. *Am J Physiol* **259:** G524–G529, 1990.
6. Aw TY, Wierzbicka GT and Jones DP, Oral glutathione increases tissue glutathione *in vivo*. *Chem-Biol Interact* **80:** 89–97, 1991.
7. Meister A and Anderson ME, Glutathione. *Annu Rev Biochem* **52:** 711–751, 1983.
8. Fonteles MC, Pillion DJ, Jeske AH and Leibach FH, Extraction of glutathione by the isolated perfused rabbit kidney. *J Surg Res* **21:** 169–174, 1976.
9. Griffith OW and Meister A, Glutathione: Interorgan translocation, turnover, and metabolism. *Proc Natl Acad Sci USA* **76:** 5606–5610, 1979.
10. Häberle D, Wahlländer A and Sies H, Assessment of the kidney function in maintenance of plasma glutathione concentration and redox state in anesthetized rats. *FEBS Lett* **108:** 335–340, 1979.
11. Anderson ME, Bridges RJ and Meister A, Direct evidence for inter-organ transport of glutathione and that the non-filtration mechanism for glutathione utilization involves γ-glutamyl transpeptidase. *Biochem Biophys Res Commun* **96:** 848–853, 1980.
12. Ormstad K, Låstbom T and Orrenius S, Evidence for different localization of glutathione oxidase and γ-glutamyltransferase activities during extracellular glutathione metabolism in isolated perfused kidney. *Biochim Biophys Acta* **700:** 148–153, 1982.
13. Rankin BB and Curthoys NP, Evidence for renal paratubular transport of glutathione. *FEBS Lett* **147:** 193–196, 1982.
14. Rankin BB, Wells W and Curthoys NP, Rat renal paratubular transport and metabolism of plasma [^{35}S]glutathione. *Am J Physiol* **249:** F198–F204, 1985.
15. Lash LH and Jones DP, Transport of glutathione by renal basal-lateral membrane vesicles. *Biochem Biophys Res Commun* **112:** 55–60, 1983.
16. Lash LH and Jones DP, Renal glutathione transport: Characteristics of the sodium-dependent system in the basal-lateral membrane. *J Biol Chem* **259:** 14508–14514, 1984.
17. Hagen TM, Aw TY and Jones DP, Glutathione uptake and protection against oxidative injury in isolated kidney cells. *Kidney Int* **34:** 74–81, 1988.
18. Griffith OW and Meister A, Translocation of intracellular glutathione to membrane-bound γ-glutamyl transpeptidase as a discrete step in the γ-glutamyl cycle. *Proc Natl Acad Sci USA* **76:** 268–272, 1979.
19. Inoue M and Morino Y, Direct evidence for the role of the membrane potential in glutathione transport by renal brush-border membranes. *J Biol Chem* **260:** 326–331, 1985.
20. Visarius TM, Putt DA, Schare JM, Pegouske DM and Lash LH, Pathways of glutathione metabolism and transport in isolated proximal tubular cells from rat kidney. *Biochem Pharmacol* **52:** 259–272, 1996.
21. Guder WG and Ross BD, Enzyme distribution along the nephron. *Kidney Int* **26:** 101–111, 1984.
22. Mohandas J, Marshall JJ, Duggin GG, Horvath JS and Tiller DJ, Differential distribution of glutathione and glutathione-related enzymes in rabbit kidney: Possible implications in analgesic nephropathy. *Biochem Pharmacol* **33:** 1801–1807, 1984.
23. Lash LH and Tokarz JJ, Oxidative stress in isolated rat renal proximal and distal tubular cells. *Am J Physiol* **259:** F338–F347, 1990.
24. Lash LH and Woods EB, Cytotoxicity of alkylating agents in isolated rat kidney proximal tubular and distal tubular cells. *Arch Biochem Biophys* **286:** 46–56, 1991.
25. Lash LH, Tokarz JJ, Woods EB and Pedrosi BM, Hypoxia and oxygen dependence of cytotoxicity in renal proximal tubular and distal tubular cells. *Biochem Pharmacol* **45:** 191–200, 1993.
26. Lash LH, Tokarz JJ, Chen Z, Pedrosi BM and Woods EB, ATP depletion by iodoacetate and cyanide in renal distal tubular cells. *J Pharmacol Exp Ther* **276:** 194–205, 1996.
27. Jones DP, Sundby G-B, Ormstad K and Orrenius S, Use of isolated kidney cells for study of drug metabolism. *Biochem Pharmacol* **28:** 929–935, 1979.
28. Lash LH and Tokarz JJ, Isolation of two distinct populations of cells from rat kidney cortex and their use in the study of chemical-induced toxicity. *Anal Biochem* **182:** 271–279, 1989.
29. Fariss MW and Reed DJ, High-performance liquid chromatography of thiols and disulfides: Dinitrophenyl derivatives. *Methods Enzymol* **143:** 101–109, 1987.
30. Lash LH and Jones DP, Uptake of the glutathione conjugate *S*-(1,2-dichlorovinyl)glutathione by renal basal-lateral membrane vesicles and isolated kidney cells. *Mol Pharmacol* **28:** 278–282, 1985.
31. Scott RD and Curthoys NP, Renal clearance of glutathione measured in rats pretreated with inhibitors of glutathione metabolism. *Am J Physiol* **252:** F877–F882, 1987.
32. Huener A, Schwegler JS and Silbernagl S, Renal tubular transport of glutathione in rat kidney. *Pflugers Arch* **414:** 551–557, 1989.
33. Parks LD, Zalups RK and Barfuss DW, Heterogeneity of glutathione synthesis and secretion in the proximal tubule of the rabbit. *Am J Physiol* **274:** F924–F931, 1998.
34. Lash LH, *In vitro* methods of assessing renal damage. *Toxicol Pathol* **26:** 33–42, 1998.
35. Lash LH and Anders MW, Uptake of nephrotoxic *S*-conjugates by isolated rat renal proximal tubular cells. *J Pharmacol Exp Ther* **248:** 531–537, 1989.

36. Lash LH and Zalups RK, Activities of enzymes involved in renal cellular glutathione metabolism after uninephrectomy in the rat. *Arch Biochem Biophys* **309:** 129–138, 1994.
37. Lash LH and Zalups RK, Alterations in renal cellular glutathione metabolism after *in vivo* administration of a subtoxic dose of mercuric chloride. *J Biochem Toxicol* **11:** 1–9, 1996.
38. Dantzler WH and Wright SH, Renal tubular secretion of organic anions. *Adv Drug Del Rev* **25:** 217–230, 1997.
39. Miller DS and Pritchard JB, Dual pathways for organic anion secretion in renal proximal tubule. *J Exp Zool* **279:** 462–470, 1997.
40. Ullrich KJ, Renal transporters for organic anions and organic cations. Structural requirements for substrates. *J Membr Biol* **158:** 95–107, 1997.
41. Chen Z and Lash LH, Evidence for mitochondrial uptake of glutathione by dicarboxylate and 2-oxoglutarate carriers. *J Pharmacol Exp Ther* **285:** 608–618, 1998.
42. Lash LH, Visarius TM, Sall JM, Qian W and Tokarz JJ, Cellular and subcellular heterogeneity of glutathione metabolism and transport in rat kidney cells. *Toxicology* **130:** 1–15, 1998.