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Glutathione uptake and protection against oxidative injury in isolated kidney cells

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Glutathione uptake and protection against oxidative injury in isolated kidney cells. Analysis with radiotracer and high performance liquid chromatography techniques showed that glutathione (GSH) is transported intact into cells primarily of proximal tubule origin. Characteristics of GSH uptake were the same as previously reported for basal-lateral membrane vesicles, namely, uptake was Na^+-dependent, inhibited by γ-glutamylglutamate and/or probenecid, and not inhibited by cysteinylglycine or the constituent amino acids. Studies with inhibitors of γ-glutamyltransferase (acivicin) and γ-glutamylcysteine synthetase (buthionine sulfoximine) showed that GSH uptake, degradation and resynthesis are independent processes. The GSH uptake rate with 1 mM GSH was approximately three-fold greater than the GSH synthetic rate with 1 mM amino acids. To examine whether uptake of GSH can supplement synthesis to protect against injury, we incubated cells with a toxic concentration of *t*-butylhydroperoxide with or without GSH or its constituent amino acids. Although amino acids provided significant protection, GSH provided greater protection (cells with *t*-butylhydroperoxide plus GSH were not significantly different from cells alone). This protection by GSH was eliminated by γ-glutamylglutamate or probenecid, indicating that GSH uptake was required for the protection seen. Protection was also eliminated when the GSSG reductase/GSH peroxidase system was inhibited by bischloronitrosourea (BCNU), indicating that GSH transport affords protection by maintaining GSH levels in the cell. Thus, intact GSH is transported into isolated proximal tubule cells by a Na^+-dependent system, and this transported GSH can be used to supplement endogenous synthesis and GSSG reduction to protect cells against oxidative injury.

Because the kidneys function in toxin and waste elimination, the epithelium of the renal tubule can be exposed to a variety of toxic compounds. The epithelial cells of the renal tubule have a high concentration of glutathione (GSH) [1], a tripeptide involved in detoxication. GSH reacts with electrophilic compounds and serves as a reductant for selenium-dependent and independent peroxidases [2]. Studies with a variety of cell types have shown that decreases in GSH increase the sensitivity to oxidative and chemical injury.

Recently, our laboratory has established that isolated cells from the intestine and lungs are substantially protected against *t*-butylhydroperoxide-, menadione-, or paraquat-induced toxicity when incubated with GSH, either at 1 mM or at a physiological plasma concentration (20 μM) [3, 4]. For it to be an effective cellular protectant, GSH had to be transported into these cells.

In isolated plasma membrane vesicles from rat kidney cortex, GSH is taken up by a Na^+-dependent, electrogenic mechanism in the basal-lateral membrane, but is not transported by this mechanism in brush border membranes [5, 6]. GSSG, γ-glutamylglutamate and a glutathione conjugate were also transported in basal-lateral membrane vesicles by a Na^+-dependent, electrogenic mechanism, and transport of these compounds was competitive with GSH transport. Incubations with a fivefold excess concentration of Cys, Gly, or Cys-Gly did not inhibit transport while a fivefold excess of Glu gave only 25% inhibition. Thus, the basal-lateral transport system appears to be specific for the γ-glutamyl moiety and relatively non-specific for other portions of the GSH molecule. Calculations of the energy available from the plasma membrane potential and sodium gradient in the proximal tubule cell indicate that sufficient energy is available to drive GSH uptake against the substantial concentration gradient that occurs from the blood plasma to cell cytosol [6].

An electrogenic GSH transport system has also been described in renal brush-border membrane vesicles [7]. This transporter has characteristics compatible with a role in GSH efflux from the cell into the tubular lumen, and recent studies [8] have indicated that kidneys secrete GSH into the lumen. Thus, current evidence indicates that both uptake and efflux can occur in the kidney. This transport may be important both in regulation of renal and organismic GSH homeostasis.

In the current study, we examined whether isolated kidney cells can take up intact GSH. The results show that such transport does occur and can function to supplement endogenous synthesis of GSH to protect against injury by the oxidant, *t*-butylhydroperoxide.

Methods

Glutathione (GSH), glutathione disulfide (GSSG), 1-fluoro-2, 4-dinitrobenzene, collagenase Types I and IV, Percoll, probenecid, HEPES [N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid], heparin, and γ-L-glutamyl-L-glutamate were purchased from Sigma, St. Louis, Missouri, USA. Ophthalmic acid was purchased from Bachem (Torrance, California, USA). Acivicin [L-(αS-5S)-α-amino-3-chloro-4,5-dihydro-5-isoxazole acetic acid] was from Upjohn Co., Kalamazoo, Michigan, USA. [Glycine-2-^{3}H]GSH (specific activity, 1.1 Ci/mmol), ^{14}C-glycine (50.3 mCi/mmol) and ^{14}C-Cys-Cys (306 mCi/mmol) were

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purchased from New England Nuclear, Boston, Massachusetts, USA. Carmustine [N,N-bis(2-chloroethyl)-N-nitrosourea; BCNU] was obtained from the Emory University Hospital. All other chemicals used were reagent grade.

Male white rats (Sprague-Dawley outbred albino, King Animal Laboratories, Oregon Wisconsin, USA) weighing between 175 and 250 g were anesthetized by injection of Nembutal (0.1 ml/0.1 kg) i.p. and killed after cannulation by cutting through the diaphragm. Isolated kidney cells were obtained by a collagenase perfusion technique as previously described [9]. This preparation contains a mixture of cell types, of which 70 to 80% are of proximal tubular origin [10]. Cell viability was assessed by the ability of the cells to exclude 0.2% trypan blue. Initial viability was 80 to 95%. Cell concentrations were quantified using a hemacytometer.

Cell viability studies

Isolated cells (10^6 cells/ml) were suspended in Krebs-Henseleit medium (pH 7.4) that was preequilibrated with 95% O_2/5% CO_2 and supplemented with 12.5 mM HEPES, 7 mM glutamate, and 5 mM glucose. All incubations were performed in a gyrotory waterbath at 37°, except as indicated. In some studies, γ-glutamyltransferase activity was inhibited by preincubation of the cell suspension with 0.25 mM acivicin [11] for 20 minutes prior to the start of the experiment. In the viability studies, agents tested for their ability to protect renal epithelial cells against *t*-butylhydroperoxide-mediated cell death (1 mM and 20 μM GSH, 0.5 mM GSSG, 0.5 mM dithiothreitol [DTT], or 1 mM each of cysteine, glutamate, and glycine) were added to the cell suspension 10 minutes prior to addition of 150 μM *t*-butylhydroperoxide. When the physiological GSH concentration was used (20 μM), GSH was added to the cell suspension every 30 minutes throughout the experimental time course to maintain the concentration. When 50 μM BCNU (in dimethylformamide) was present, it was added 30 minutes prior to the start of the experiment. When probenecid (1 mM) and/or γ-glutamylglutamate (10 mM) was used, these compounds were added five minutes prior to addition of GSH.

Glutathione uptake studies

Cells (10^6 cells/ml) were pretreated for 30 minutes with acivicin to inhibit γ-glutamyltransferase, and the experiments were initiated by addition of 1 mM ^{3}H-GSH (0.1 mCi/mmol). Aliquots (0.5×10^6 cells/aliquot) were layered onto 10% Percoll in Krebs-Henseleit medium in microcentrifuge tubes at the times indicated. The tubes were centrifuged for 30 seconds, the supernatants aspirated, and the pellets washed by resuspending in Krebs-Henseleit medium and recentrifuging for 30 seconds. After removing the supernatant, the cells were lysed and protein was precipitated by addition of 150 μl of 30% (wt/vol) trichloroacetic acid. Radioactivity in the supernatant was determined by liquid scintillation counting.

To test for intact GSH transport, cell lysates were derivatized with 40 mM iodoacetic acid and 1-fluoro-2,4-dinitrobenzene [1.5% (vol/vol) in absolute ethanol] as described by Reed et al [12]. The dinitrophenyl derivatives were separated by high performance liquid chromatography (HPLC) on a 5 μm Aminospheri-5 column (Brownlee Laboratory, Santa Clara, California, USA) and detected at 365 nm. GSH in the lysates was quantified relative to standards by integration.

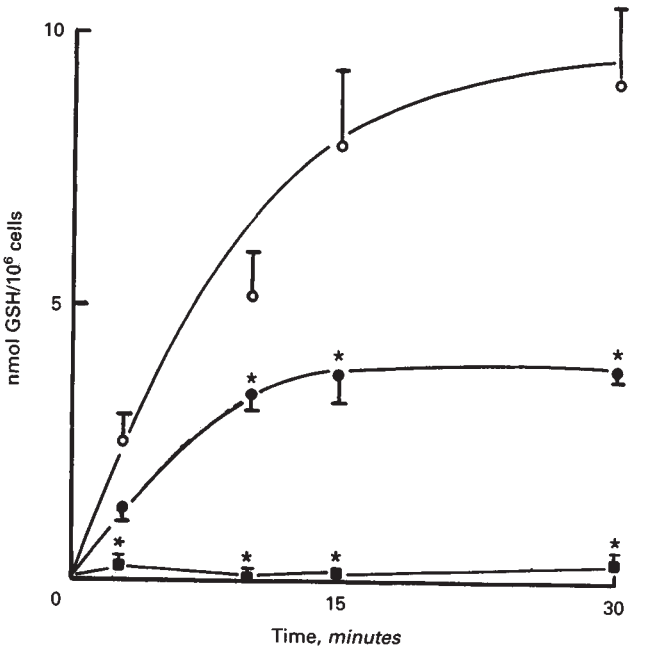

Fig. 1. *Uptake of GSH by isolated renal proximal tubule epithelial cells.* Incorporation of ^{3}H-GSH into cells was quantified by scintillation counting of the cellular extracts. Aliquots were taken at 3, 10, 15, and 30 min. The initial rate of GSH uptake was 1.1 nmol/10^6 cells per min. Symbols are: (○) Na^+; (●), no Na^+; (■) cells under anaerobic conditions. Values are given as mean ± SE for 4 cell preparations. Values for Na^+-free and anaerobic incubations were significantly different ($P < 0.05$) from corresponding Na^+ containing and aerobic incubations at time points indicated by an asterisk.

The dependence of GSH transport on Na^+ was assessed by use of Na^+-free Krebs-Henseleit medium where an equimolar concentration of choline Cl was substituted for NaCl. The ability of γ-glutamyl compounds to inhibit transport was examined by preincubation with 10 mM γ-glutamylglutamate in the cell suspension five minutes prior to ^{3}H-GSH addition. Probenecid (1 mM), a known organic anion transport inhibitor [13] and inhibitor of GSH transport in basal-lateral membrane vesicles, was also added to isolated cell suspensions to assess whether this affected transport.

For anaerobic experiments, cells (1.0×10^6 cells/ml) were suspended in modified Krebs-Henseleit medium that had been deoxygenated by bubbling with 100% argon. Oxygen concentration was monitored by an oxygen electrode (Yellow Springs Instruments, Model 53) and, after the cells became anaerobic, a radiolabeled GSH solution was added and aliquots were taken as described above.

Results

Transport of GSH

To examine GSH uptake in kidney cells, we first treated cells with acivicin to inhibit γ-glutamytransferase to minimize artefacts due to GSH breakdown. Acivicin-treated cells (10^6/ml) incubated with 1 mM [glycine-2-^{3}H]GSH showed an increase in radiolabel that approached a steady state after 20 minutes (Fig. 1); the initial rate of transport based upon the specific activity of added GSH was 1.1 nmol/10^6 cells per minute. HPLC of cellular

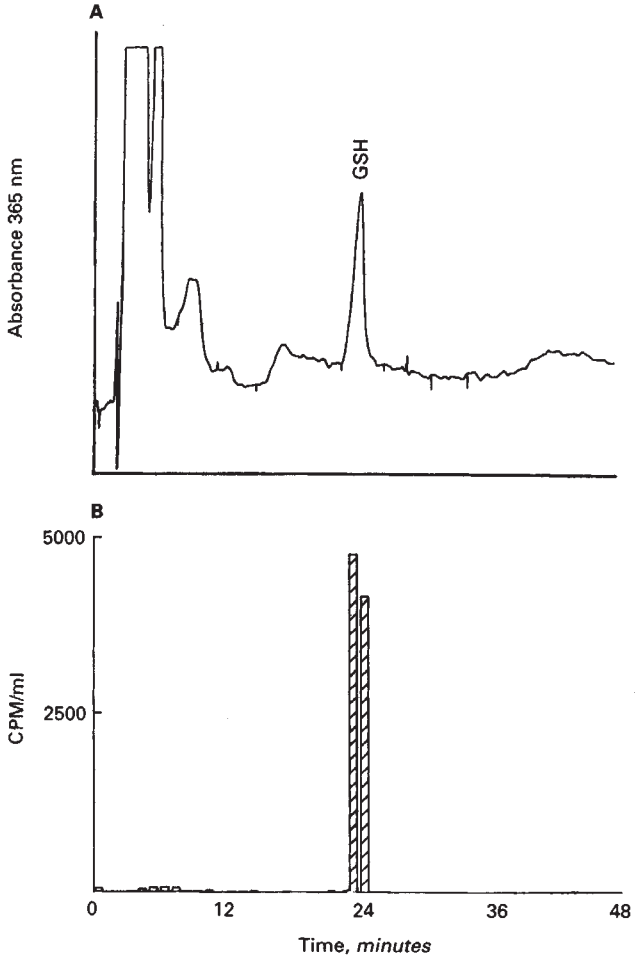

Fig. 2. *Analysis of GSH uptake by HPLC.* Supernatants from trichloroacetic acid (10%) precipitates of cells were derivatized according to Reed et al [12]. **A.** Typical HPLC elution profile of DNP-derivatized cellular extracts is shown (15 min time point). GSH retention time = 23.3 min. **B.** HPLC elution profile of ^{3}H-radiolabel. Eluent from the HPLC was collected (1 ml/min per fraction tube) and quantified by scintillation counting. Greater than 90% of the radiolabel comigrated with the same retention time as GSH.

GSH content (Fig. 2A) showed that the concentration increased from 2 to 18 nmol/10^6 cells within 30 minutes. Analysis of radiolabel content at 15 minutes showed that 80% or more of the radiolabel co-migrated with the GSH peak (Fig. 2B). The rest of the radiolabel co-migrated with GSH-Cys (up to 15%) or Gly (up to 5%).

To compare the rate of uptake to GSH synthesis, we determined the rate of GSH synthesis with 1 mM concentrations of amino acid precursors containing ^{14}C-cystine or ^{14}C-glycine. The rate of GSH formation (0.15 to 0.28 nmol/10^6 cells per min; Table 1) corresponds with previously reported rates (0.33 nmol/10^6 cells per min) [10], and shows that the rate of uptake under the conditions of our experiment (1.1 nmol/10^6 cells per min) is greater than the rate of GSH synthesis.

Since the radiolabel of GSH used in the transport studies was located in the glycine moiety, experiments were performed to test whether exchange of ^{14}C-Gly with the Gly moiety of GSH could account for the radiolabeled GSH that was accumulated

Table 1. Rate of GSH synthesis in isolated renal proximal tubule epithelial cells

Conditions	Rate of GSH synthesis *nmol/10^6 cells per min*	
	(−) BSO	(+) BSO
1 mM amino acids (^{14}C-Cys)	0.15 ± 0.04	0.01 ± 0.001
1 mM amino acids (^{14}C-Gly)	0.18 ± 0.04	0.04 ± 0.01
1 mM amino acids (^{14}C-Gly) + 1 mM GSH	0.28 ± 0.06	0.06 ± 0.01

Cells (10^6/ml) were incubated with amino acids at the indicated concentration and radiolabeled species. After 15 min, cell lysates were derivatized for HPLC. All numbers given are the average ± SE of 3 experiments.

in the cells. We added 1 mM each of amino acid precursors, including ^{14}C-Gly (0.3 μCi/μmol), in the presence of 1 mM cold GSH and monitored the appearance of radiolabeled GSH by HPLC. Results show that the rate of appearance of radiolabeled GSH into cell lysates was 0.28 nmol/10^6 cells per minute (Table 1). This corresponds to the rate of synthesis without cold GSH and is insufficient to account for the rate of radiolabeled GSH appearance found in the transport studies. In all the studies with radiolabeled cysteine as the precursor, the inhibitor of GSH synthesis buthionine sulfoximine (BSO) [14] gave greater than 90% inhibition of GSH synthesis. Thus, these results confirm that transport of intact GSH is necessary to account for the radiolabel associated with GSH in the previous experiments.

To determine whether GSH transport occurred in the presence of γ-glutamyltransferase activity, experiments were performed as above (Figs. 1, 2) but without acivicin treatment. Again HPLC analysis showed that GSH increased over time and that between 60 and 70% of the radiolabel co-migrated with the GSH peak. The remaining radioactivity was distributed in unresolved fractions probably representing glycine, cysteinylglycine, and the mixed disulfide of Cys and GSH. No radiolabeled GSSG was detected. Addition of 1 mM BSO, a concentration that inhibits GSH synthesis by 90%, gave only 30% decrease in the rate of radiolabeled GSH appearance in cells when cells were incubated with ^{3}H-GSH. From these results, it is clear that GSH is transported intact by renal proximal tubule epithelial cells; moreover, GSH transport occurs even in the presence of γ-glutamyltransferase activity.

Previous studies with basal-lateral membrane vesicles from kidney demonstrated that GSH transport is a Na^+-dependent and electrogenic process [6]. We examined the Na^+ dependence of GSH transport in isolated cells by suspending them in a Na^+-free Krebs-Henseleit medium. Results show that GSH transport in the absence of Na^+ is less than 50% of the Na^+-containing controls (Fig. 1). Thus, much of the GSH tranpsort is Na^+-dependent. Assuming a volume of 3 μl/10^6 cells, uptake occurs from 1 mM outside to 6 mM inside and has the characteristics of secondary active transport. Consistent with this, incubation of cells under anaerobic conditions resulted in nearly complete inhibition of uptake (Fig. 1).

Because the transport of GSH in renal basal-lateral membrane vesicles is inhibited by other γ-glutamyl compounds or probenecid [6], γ-glutamylglutamate (10 mM), ophthalmic acid (10 mM) or probenecid (1 mM) was added along with the ^{3}H-GSH solution to test for inhibition of cellular uptake. The

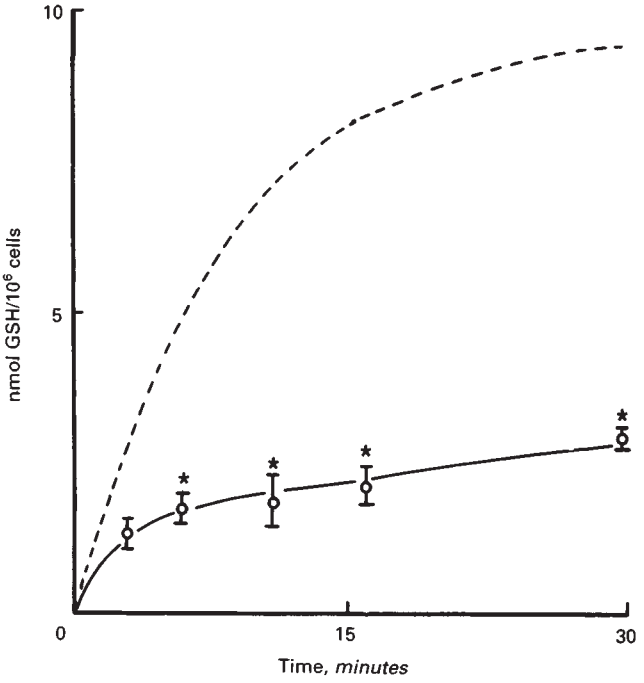

Fig. 3. *γ-Glutamylglutamate inhibits uptake of GSH.* Cells (1.0×10^6 cells/ml) were pretreated with 10 mM γ-glutamylglutamate 10 min prior to addition of the ^{3}H-GSH solution. Aliquots were taken at times indicated and quantified as in Fig. 1. Symbols represent GSH uptake in the presence of γ-glutamylglutamate. The dashed line represents the uptake of GSH as shown in Fig. 1. Data are given as the mean ± SE for three cell preparations. Values were significantly different ($P < 0.05$) from controls (Fig. 1) at all time points indicated by an asterisk. Identical results were obtained with 10 mM ophthalmic acid. Steady-state accumulations were slightly higher (about 50% of control values) with 1 mM probenecid.

rate of uptake was inhibited by about 60% (Fig. 3, Table 2) by any of these compounds. In other experiments, cells were incubated with a 10 mM solution of Glu, Cys, Gly, or Cys-Gly in addition to the ^{3}H-GSH solution (Table 2). None of these compounds inhibited GSH transport substantially, indicating that the amino acid carriers in the proximal tubule are not responsible for GSH transport. These results indicate that GSH is recognized by a transport mechanism that is similar or identical to that previously described for isolated basal-lateral membrane vesicles from kidney.

Protection of GSH

Since GSH plays a key role in cellular detoxication mechanisms, we examined whether transported GSH could aid in the protection of renal proximal tubule cells against chemically induced oxidative injury. Addition of 150 μM *t*-butylhydroperoxide to cell suspensions (10^6 cells/ml) caused a dramatic decrease in cell viability in two hours (Fig. 4A). While the mechanism of toxicity has not been studied in the kidney, this compound is known to cause hepatocellular injury by a process involving the oxidation of intracellular GSH [15], perturbations of Ca^{+2} homeostasis [16], and oxidation of cellular and mitochondrial pyridine nucleotides [17]. Addition of 1 mM GSH along with 150 μM *t*-butylhydroperoxide resulted in preservation of cell viability to values comparable to control incubations. Thus, exogenous GSH substantially protected these cells against *t*-butylhydroperoxide-mediated cell death.

Table 2. The effect of amino acid constituents, γ-glutamyl analogs and probenecid on initial GSH transport rate

Substance added	*N*	% of Control
Control	4	—
Glutamate *10 mM*	3	100
Cysteine *10 mM*	3	107
Cysteinylglycine *10 mM*	2	97
Glycine *10 mM*	3	89
γ-Glutamylglutamate *10 mM*	4	40
Ophthalmic Acid *10 mM*	4	40
Probenecid *1 mM*	4	50

The controls for these experiments were done in the absence of Glu. The rate of GSH transport was 1.0 nmol/10^6 cells per min, approximately equal to the rate in the presence of 7 mM GluGlu.

To assess whether the physiological concentration in the plasma [18, 19] could provide protection, cells were incubated with 150 μM *t*-butylhydroperoxide and 20 μM GSH (Fig. 4B). Although there was a slight decline in viability after two hours, this was not statistically different ($P < 0.05$) from controls (Fig. 4B). Therefore, these results show that protection can occur at a GSH concentration in the physiological range for blood plasma.

To compare protection by exogenous GSH with that provided by endogenous GSH synthesis, equimolar concentrations (1 mM) of cysteine, glutamate, and glycine were added to cell suspensions, with and without *t*-butylhydroperoxide. These amino acids provided significant protection but were less than half as effective as GSH alone (Fig. 5A).

Preincubation of the cells with 1 mM BSO inhibits synthesis of GSH from the amino acid precursors [14]; this treatment also provides an approach to determine whether amino acids could offer any protection to renal proximal tubule cells. Results show that no statistically significant protection from *t*-butylhydroperoxide occurred with the amino acids when 1 mM BSO was included (Fig. 5B); the viability of cells declined to 28% after two hours, as compared to 18% in those cells with only *t*-butylhydroperoxide added (Fig. 5B).

Studies with basal-lateral membrane vesicles indicated that GSSG may also be transported into proximal cells, but at a much slower rate [6]. Therefore, GSSG could conceivably protect proximal cells if it were transported and then subsequently reduced intracellularly. We tested this by addition of 0.5 mM GSSG to cells along with *t*-butylhydroperoxide. Results show that GSSG gave a slight protection to proximal cells (Fig. 5B). Thus, although this protection is statistically significant, the reduced form of GSH is necessary for optimal protection to occur.

Loss of protection

The above data show that exogenous GSH protects kidney cells against *t*-butylhydroperoxide injury; however, because both GSH and *t*-butylhydroperoxide are supplied exogenously to the cells, it is possible that the protection seen may be due to reaction of GSH with *t*-butylhydroperoxide in the medium. To address this possibility, we used probenecid, γ-glutamylglutamate, or ophthalmic acid to block GSH uptake [6, present study]. If GSH protection were a consequence of extracellular reac-

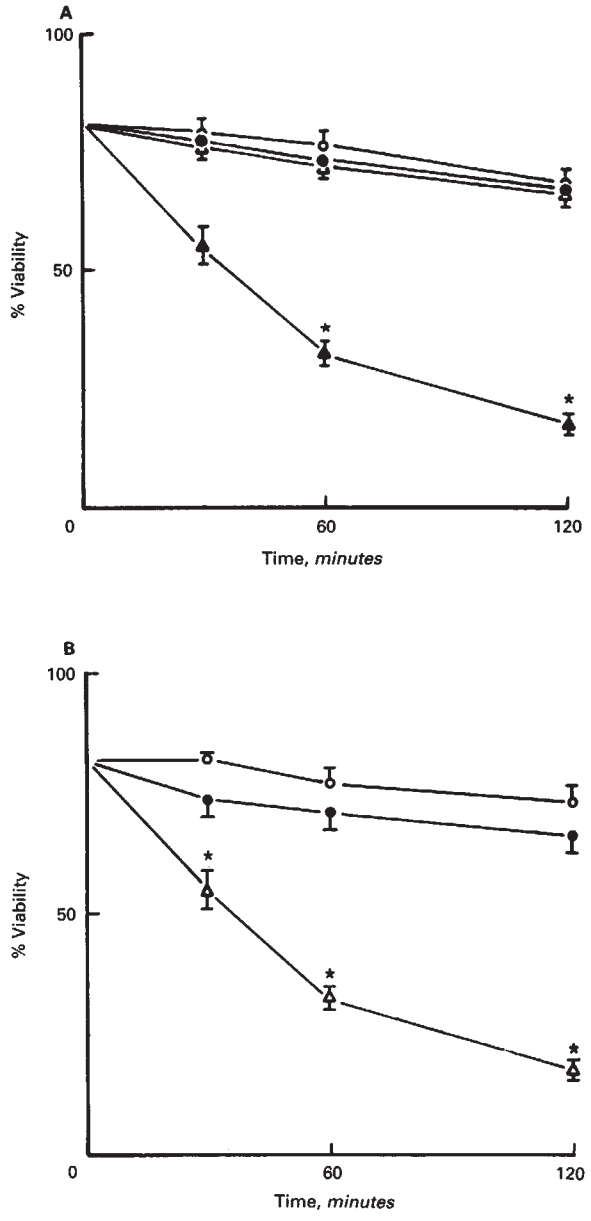

Fig. 4. *Protection of renal proximal tubule epithelial cells from t-butylhydroperoxide-induced injury by exogenous GSH.* **A.** Cells (1.0×10^6 cells/ml) were suspended in modified Krebs-Henseleit medium and, where appropriate, 1 mM GSH was added 10 min prior to the start of the experiment. *t*-Butylhydroperoxide (150 μM, final concentration) was added at the beginning of the experiment. Symbols are: (○) control; (●) cells + 1 mM GSH; (△) cells + 1 mM GSH + 150 μM *t*-butylhydroperoxide; (▲) cells + 150 μM *t*-butylhydroperoxide. **B.** Cell suspensions were treated as above except that 20 μM GSH was added. GSH was added every 30 min to maintain the extracellular concentration. Aliquots were taken at times indicated, and viability was assessed by 0.2% trypan blue exclusion. Symbols are: (○) cells + 20 μM GSH; (●) cells + 20 μM GSH + 150 μM *t*-butylhydroperoxide; (△) cells + 150 μM *t*-butylhydroperoxide. Averages of at least 4 experiments ± SE are given. Values for incubation with *t*-butylhydroperoxide were significantly different ($P < 0.05$) from control and *t*-butylhydroperoxide + GSH as indicated by an asterisk.

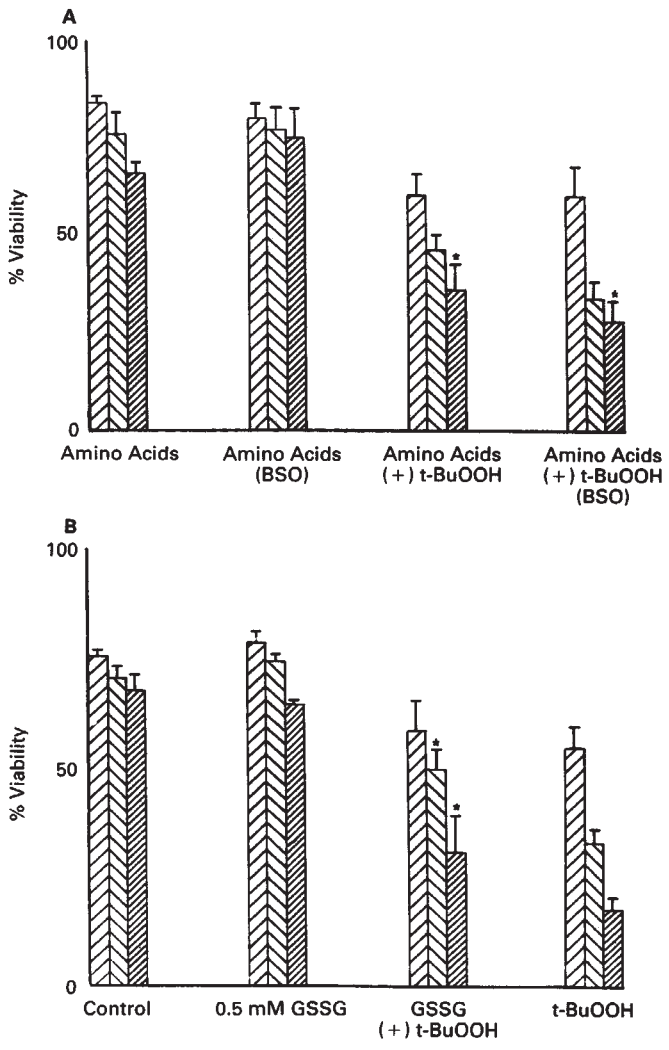

Fig. 5. *Effect of amino acid constituents of GSH or GSSG on protection renal proximal epithelial cells from t-butylhydroperoxide-induced injury.* **A.** 1 mM concentrations of glutamate, cysteine, and glycine were added to cell suspensions 10 min prior to addition of 150 μM *t*-butylhydroperoxide and viability was assessed by trypan blue exclusion. To test whether amino acids themselves could offer any protection, 1 mM BSO was added to some incubations to inhibit GSH synthesis. **B.** Cell suspensions were treated as in Fig. 3 except that 0.5 mM GSSG was added instead of reduced GSH. Symbols are: ▨, 30 min; ▧, 60 min; ▩, 120 min. Mean ± SE for 3 preparations are given. Statistical significance ($P < 0.05$) is indicated by an asterisk.

tion, protection would still be seen. However, if protection were due to transport of GSH by the cells, then protection would be lost. Cells were incubated with γ-glutamylglutamate and the organic anion transport inhibitor, probenecid, either in separate incubations or together. Results show that protection was lost in cell suspensions with the inhibitors (Fig. 6), but the inhibitors alone did not cause loss of cell viability. Thus, GSH protection of isolated renal cells does not appear to be a consequence of extracellular reaction of GSH with *t*-butylhydroperoxide but requires that GSH is transported to exert its protective effects.

To examine whether an intact GSH peroxidase/GSSG reductase system is required for the protection seen by exogenous GSH, cells were treated with BCNU at a concentration that inhibits GSSG reductase but has little effect on cell viability. The results show that BCNU prevents protection by GSH

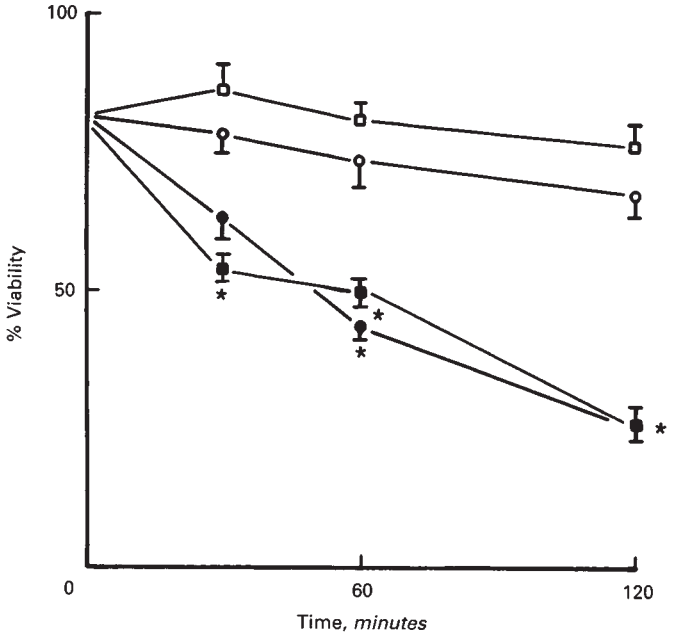

Fig. 6. *Exogenous GSH fails to provide protection of proximal tubule epithelial cells when cells are pretreated with inhibitors of GSH transport.* To appropriate cell suspensions, 10 mM γ-glutylglutamate, 1 mM probenecid, or both were added 5 min prior to addition of GSH and the experiment was conducted as in Fig. 3. Symbols are: (□) cells + 1 mM GSH + 10 mM γ-glutamylglutamate + 1 mM probenecid; (○) cells + 1 mM GSH + 10 mM γ-glutamylglutamate; (■) cells + 1 mM GSH + γ-glutamylglutamate + 1 mM probenecid + 150 μM *t*-butylhydroperoxide; (●) cells + 1 mM GSH + γ-glutamylglutamate + 150 μM *t*-butylhydroperoxide. Mean ± SE for 3 cell preparations are given. Values of cells + GSH + γ-glutamylglutamate + *t*-butylhydroperoxide are not significantly different ($P < 0.05$) from cells with *t*-butylhydroperoxide alone, but each indicated by an asterisk is significantly different ($P < 0.05$) from controls or cells + *t*-butylhydroperoxide + GSH.

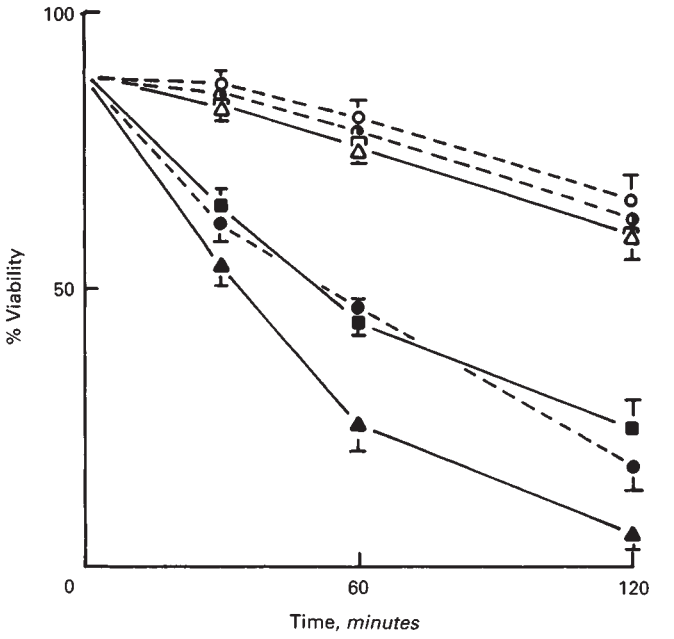

Fig. 7. *Effect of BCNU (50 μM) on GSH-dependent protection against t-butylhydroperoxide-induced cell death.* BCNU was added to acivicin-treated cells (10^6 cells/ml) 20 min prior to addition of 1 mM GSH. The experiment was then carried out as in Fig. 4. Symbols are: (○) cells + 1 mM GSH; (△) cells + 50 μM BCNU; (◑ cells + 1 mM GSH + 50 μM BCNU; (□) cells + 1 mM GSH + 150 μM *t*-butylhydroperoxide; (●) cells + 150 μM *t*-butylhydroperoxide; (■) cells + 150 μM *t*-butylhydroperoxide + 1 mM GSH + 50 μM BCNU; (▲) cells + 150 μM *t*-butylhydroperoxide + 50 μM BCNU. Time points represented by closed symbols were significantly different ($P < 0.05$) from their respective controls (open circles).

against *t*-butylhydroperoxide-induced damage (Fig. 7). BCNU pretreatment also caused a further loss in viability of those cells treated with *t*-butylhydroperoxide without GSH and caused a small (statistically significant) loss in control cell viability. Thus, exogenous GSH protects renal epithelial cells by a mechanism involving uptake of intact GSH and function of the GSSG reductase/GSH peroxidase system.

Discussion

Previous studies have shown that GSH efflux occurs from the liver into the blood [20, 21] and efflux can be enhanced by certain hormones [22]. Since this discovery, many investigations have centered on the physiological importance of plasma-derived GSH. It is known that the kidney is the major organ for GSH and GSH-S conjugate clearance from the plasma [23], with as much as 80% of plasma GSH turnover occurring in the kidney. GSH degradation was once thought to solely account for this turnover, due to an abundance of γ-glutamyltransferase found in the brush border of renal proximal tubule epithelial cells [24, 25]. However, glomerular filtration alone cannot account for all the GSH extracted because only about 25% of the blood volume is filtered. Rankin and Curthoys [26] and Ormstad, Jones and Orrenius [10] suggested the possibility that a mechanism for GSH transport existed. Subsequent studies demonstrated that a Na^+-dependent, electrogenic, carrier-mediated GSH transport mechanism exists in basal-lateral membrane vesicles of the kidney [5, 6]. This transporter also accounts for the uptake of the GSH-S conjugate, S-(1,2-dichlorovinyl)GSH [27], thus providing the basis for a secretory mechanism for GSH-S conjugate clearance from the kidney. The current study gives evidence that intact GSH is transported into isolated kidney epithelial cells. Moreover, transport of intact GSH was demonstrated both in the presence of γ-glutamyltransferase or when activity was inhibited with acivicin. The uptake is Na^+-dependent and is inhibited by the same compounds that inhibit uptake in the basal-lateral vesicles. Thus, transport occurs concomitantly with hydrolysis. While the extent of direct uptake of GSH by this system in vivo remains a matter of speculation, the current results indicate that GSH transport can provide a mechanism to supplement endogenous detoxication systems to protect against chemical and oxidative injury. This process is likely to occur in vivo at plasma concentrations of GSH because 20 μM GSH also provided significant protection against *t*-butylhydroperoxide injury.

The cell preparation used for these studies has been well characterized and contains approximately 70 to 80% proximal tubule epithelial cells [9, 10]. The extent to which different cell types of the kidney transport GSH is not known, but the quantitative aspects both of the uptake and the viability studies indicate that uptake is at least a property of the proximal tubule

cells. The observation that the amino acid precursors of GSH provided little direct protection of cells against oxidative injury indicates that the amino acids themselves and/or that GSH synthesis do not provide the extent of protection that GSH uptake provides. This finding is similar to that observed with enterocytes [3] but different from the results obtained with alveolar type II cells, which showed that the amino acids provide protection equivalent to that seen with GSH [4].

The properties of GSH transport in kidney are identical to those in isolated enterocytes [3], and pulmonary alveolar type II cells [4]. It is significant that these cell types, which are substantially protected against oxidative injury by transported GSH, are all epithelial cells located in areas that are readily exposed to oxidative or xenobiotic injury. Hepatic-derived GSH in the plasma may thus provide a means to supplement intracellular GSH pools that may become depleted during chemical or oxidative injury. Oxidants such as redox cycling compounds (paraquat or menadione) or peroxides cause a rapid depletion of intracellular GSH pools [28]. Because GSH is a cosubstrate for both GSH S-transferases and GSH peroxidases, the depletion of GSH limits their activities, allowing radical producing peroxides or electrophiles to react with cellular macromolecules. Also, GSH is involved in maintaining the intracellular thiol/disulfide ratio that is vital for function of a variety of enzymes [29]. Depletion of GSH leads to alterations in this ratio and can thereby contribute to altered cellular function. Our viability studies using BCNU indicate that GSH transport protects cells by maintaining reduced GSH pools and allowing the GSH-dependent detoxication systems to continue to function. Therefore, GSH transport may be a general mechanism to maintain intracellular GSH pools in certain epithelial cells under conditions where GSH levels would otherwise be depleted.

Inoue and Morino [7] have recently found that a GSH transport mechanism exists in brush border vesicles of kidney cells. The electrogenic characteristics of this system are consistent with a role in efflux of GSH from cells into the tubular lumen. Furthermore, recent work by Scott and Curthoys has provided evidence that GSH efflux occurs from renal epithelial cells into the tubular lumen [8]. These authors speculate that GSH efflux may serve as a mechanism to bring γ-glutamyltransferase at the brush border in contact with its substrate, thereby functioning in GSH turnover. This transcellular transport of GSH may also be a way to provide GSH to the lumen to conjugate reactive species that might otherwise be concentrated in and toxic to the urinary tract.

Finally, this study provides a rationale for the possible use of GSH as a direct therapeutic agent to protect against ischemia and reperfusion injury, radiation damage, chemical toxicity, or chemical-induced carcinogenesis in the renal epithelium. Paller [30] found that infusion of GSH increased the cortical content of GSH and protected against renal dysfunction due to ischemia. Other studies in our laboratory have shown that GSH is transported intact from the lumen to the portal circulation in isolated perfused rat small intestine [31]. These results indicate that GSH may be taken up intact from the diet, providing a means by which GSH may be therapeutically administered to augment plasma GSH concentrations. This may be significant in those pathological conditions where hepatic GSH efflux is impaired, such as cirrhosis [32], under conditions of oxidative stress (O_2 therapy in newborns) [33] or in conditions where a decline in GSH is thought to allow deleterious oxidative processes (cataract formation in the lens) [34]. Clinical studies will be needed to assess any therapeutic benefit of GSH.

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