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Biochemistry

Transport of glutathione, as γ-glutamylcysteinylglycyl ester, into liver and kidney

(buthionine sulfoximine/radioprotection/toxicity/acetaminophen)

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ABSTRACT **Administration of γ-glutamylcysteinylglycyl monomethyl (or monoethyl) ester to mice leads to substantial increases in the levels of glutathione in the liver and kidney. Mice depleted of glutathione by treatment with buthionine sulfoximine, a potent inhibitor of γ-glutamylcysteine synthetase, exhibited about a 4-fold increase in liver and kidney glutathione levels after administration of glutathione monomethyl ester. This ester also prevented the marked decline in liver glutathione level found after giving mice acetaminophen, and it protected mice from toxicity due to this compound. The findings indicate that the monomethyl and monoethyl esters of glutathione are transported into cells and hydrolyzed to glutathione. Such esters may be useful in experimental work on glutathione metabolism and function and may provide a relatively safe method for protecting cells against damage by toxic compounds, oxygen, and radiation.**

Several studies have shown that intracellular glutathione (GSH) is of major importance in protecting cells against damage by toxic compounds, reactive oxygen compounds, and radiation (1–3). For this reason there has been interest in the factors that influence intracellular GSH synthesis and especially in ways of increasing cellular levels of GSH. Although the availability of cysteine may be a limiting factor in GSH synthesis, administration of this amino acid does not always lead to substantially increased GSH levels; cysteine is rapidly metabolized and it is also toxic, possibly in part because of its extracellular effects. Administration of compounds that are transported and converted intracellularly into cysteine, such as L-2-oxothiazolidine-4-carboxylate (4, 5), are useful in increasing cellular GSH. Another promising approach for increasing kidney GSH levels is administration of γ-glutamylcysteine (and related compounds), which are transported and stimulate GSH synthesis by providing a substrate for GSH synthetase (6). The possibility that administration of GSH itself might lead to increased cellular GSH levels has also been considered. Recent studies on the metabolism of GSH indicate that GSH is transported out of many cells, and that such export is a discrete step in GSH metabolism (2, 7, 8). One might think that GSH export would be reversible, but this does not seem to be the case. Although it is possible that some cells may take up intact GSH, there is as yet no conclusive evidence for transport of GSH into cells. Thus, the repletion of intracellular GSH in human lymphoid cells by exogenously supplied GSH was found to involve extracellular degradation of GSH, uptake of the products, and intracellular synthesis (9). The apparent uptake of GSH in the basolateral circulation of the kidney is evidently explained by cleavage of GSH to its constituent amino acids (10). No evidence for GSH uptake by the liver was obtained in studies on rats (11), and the moderate increase in kidney GSH levels observed after administration of GSH to mice may be explained by formation and transport of γ-glutamylcys(e)ine (6).

The present work was stimulated by the idea that a derivative of GSH might be more readily transported into cells than GSH itself; thus, we have begun to examine the biological properties of several simple derivatives of GSH that might be expected to be transported and converted to GSH intracellularly. Our findings on the monomethyl and monoethyl esters of GSH seem worthy of report at this time.

EXPERIMENTAL PROCEDURES

Materials. GSH, L-cysteine·HCl, L-cysteine methyl ester·HCl, and acetaminophen were obtained from Sigma. Mice [TAC:(SW)FBR], weighing 20–25 g, were obtained from Taconic Farms (Germantown, NY). The preparation of the several GSH derivatives will be given in detail in a subsequent publication. GSH monomethyl (and monoethyl) esters of GSH were obtained as the corresponding hydrochlorides essentially as described by Bergmann and Zervas (12). GSH monomethyl ester hydrochloride: Calculated for $C_{11}H_{20}O_6N_3SCl$: C, 36.9; H, 5.63; N, 11.7. Found: C, 36.7; H, 5.28; N, 10.8. NMR studies indicated that the glycine carboxyl group of GSH is esterified.

Methods. The mice were injected with solutions of the compounds indicated below in 0.15 M NaCl and adjusted to pH 7.5. The mice were killed by decapitation and the kidneys and livers were excised, rinsed in ice-cold water, blotted, weighed, and homogenized (Potter–Elvehjem) in 5 vol of 1% aqueous picric acid. After centrifugation, the supernatant solutions were analyzed for GSH.

GSH was determined by the glutathione disulfide (GSSG) reductase–dithionitrobenzoate recycling procedure essentially as described by Tietze (13) and as used previously in this laboratory. Under the conditions of this assay, addition of a large excess of GSH monomethyl (or monoethyl) ester did not significantly affect the observed rates of reaction with standard amounts of GSH. A small (≈5%) increase was noted, which may probably be ascribed to the presence of some free GSH in the GSH ester preparations (also detected by thin-layer chromatography). The findings indicate that GSH monomethyl (and monoethyl) esters are neither substrates nor inhibitors of GSSG reductase under the conditions of this assay.

RESULTS

In the course of studies on several derivatives of GSH, including the mono- and diamides, the mono- and dimethyl (and ethyl) esters, and the *N*-acetyl derivative, we noted a substantial increase in the GSH levels of the liver and kidney of mice after injection of the monoesters of GSH (i.e., γ-glutamylcysteinylglycyl ester). The findings are illustrated in Figs. 1–3, which

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Abbreviations: GSH, glutathione; GSSG, glutathione disulfide.

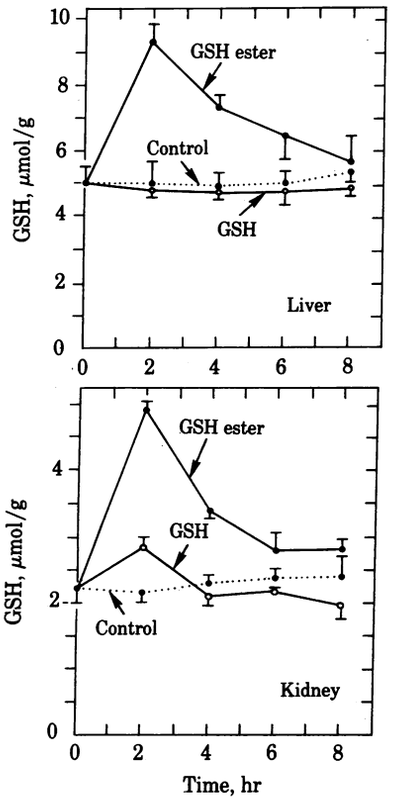

FIG. 1. Administration of GSH monomethyl ester increases GSH levels of liver and kidney of fasted mice. The mice (fasted 24 hr) were given GSH monomethyl ester or GSH (10 mmol/kg) intraperitoneally, and at the indicated intervals the tissue GSH levels were determined. Controls were given an equivalent volume of 0.15 M NaCl. Values are given as means ± SD, for groups of three animals. The effect of GSH ester on tissue levels of GSH was found to be dose dependent over the dose range thus far studied—i.e., 2.5–20 mmol/kg.

describe the effects of administration to mice of GSH monomethyl ester.

In the studies summarized in Fig. 1, giving the GSH ester led to about a doubling of the GSH levels of kidney and liver 2 hr after injection. Administration of GSH had no effect on liver GSH levels and produced a small increase in the kidney GSH level, in agreement with earlier findings (6).

In the studies described in Fig. 2, mice were pretreated with L-buthionine (*SR*)-sulfoximine (14–16), a compound that effectively inhibits γ-glutamylcysteine synthetase *in vitro* and *in vivo* (14–18) and, therefore, the synthesis of GSH. After 4 hr, the tissue levels of GSH decreased to 10–20% of the initial levels. Administration of the GSH ester led to about a 4-fold increase in the levels of GSH in the kidney and liver after 2 hr. Administration of GSH had no significant effect on the tissue GSH levels.

Mice treated with a sublethal dose of acetaminophen exhibited a marked decrease in the level of liver GSH (Fig. 3; control). No such decline in GSH levels was observed in the mice treated with acetaminophen followed by injection of GSH monomethyl ester; indeed, the GSH level of the liver increased markedly. Under these conditions, the administration of GSH, cysteine, and cysteine methyl ester affected the tissue levels of GSH much less. In another series of experiments mice were given a lethal dose of acetaminophen (5 mmol/kg) followed 1 hr later by GSH monomethyl ester (10 mmol/kg). In this study, in which 15–20 mice were used in each group and 40 mice were not treated with GSH ester, all of the untreated animals died

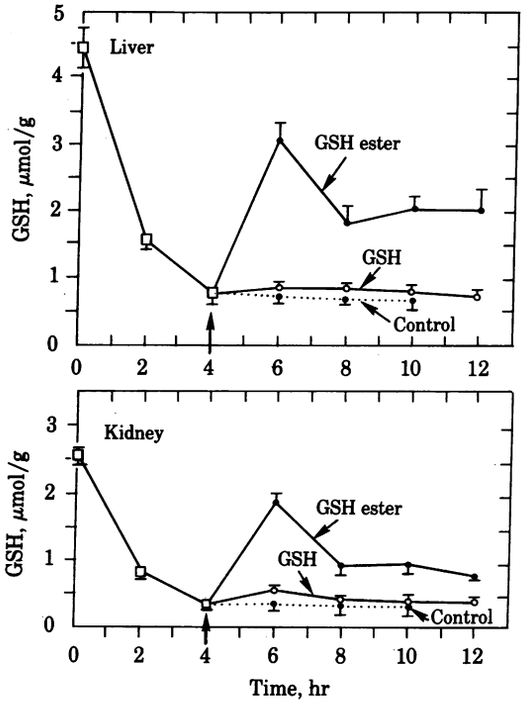

FIG. 2. Administration of GSH monomethyl ester increases GSH levels of liver and kidney of mice pretreated with buthionine sulfoximine. Mice fasted for 24 hr were injected with buthionine sulfoximine (2 mmol/kg). Four hours later (arrow), groups of mice (three) were given GSH monomethyl ester or GSH (10 mmol/kg) or saline (control) intraperitoneally; values are means ± SDs. Tissue GSH levels were determined at the indicated intervals.

within 7 days. All of the mice treated with the monomethyl ester of GSH or with the corresponding monoethyl ester survived. Partial protection was observed in mice given GSH.

DISCUSSION

The findings are most reasonably explained by transport of the administered GSH ester followed by its intracellular hydrolysis

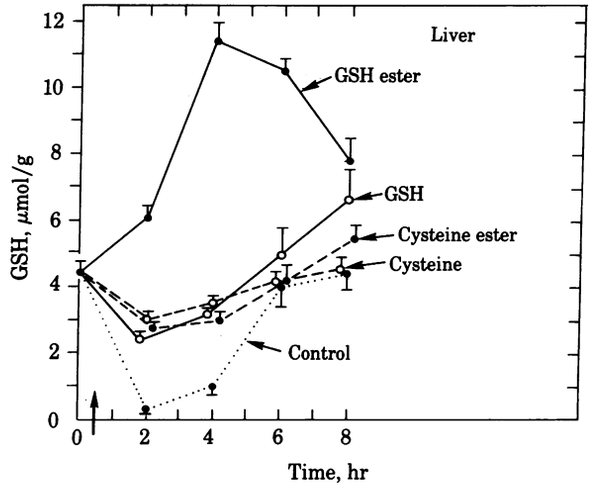

FIG. 3. Administration of GSH monomethyl ester increases GSH levels of the livers of mice previously given acetaminophen. Fasted mice were injected intraperitoneally with acetaminophen (2.5 mmol/kg), and 1/2 hr later (indicated by arrow) they were given GSH ester, GSH, L-cysteine, L-cysteine methyl ester (10 mmol/kg), or an equivalent volume of 0.15 M NaCl. Tissue GSH levels were determined at the indicated intervals on groups (three) of mice; values are means ± SDs.

to GSH. This is supported by several experiments in which GSH monomethyl ester was incubated with homogenates of liver and kidney and in which rapid hydrolysis of this compound to GSH was observed. The studies in which mice were pretreated with buthionine sulfoximine (Fig. 2) provide strong evidence for transport of GSH ester, because under these conditions synthesis of GSH from its constituent amino acids is markedly inhibited. That the GSH esters effectively protect against acetaminophen toxicity provides additional evidence for intracellular GSH formation; however, it cannot be excluded that the esters themselves may react directly with the highly reactive intermediate formed in acetaminophen metabolism.

That GSH esters are more effectively transported than GSH itself is in accord with observations made earlier by Goldman and colleagues (19–22), who found, in studies on rat liver lysosomes, that the lysosomal membrane is less permeable to free amino acids and dipeptides than to the corresponding esters, and that the esters are substrates of lysosomal esterase. Their work also demonstrated transport of trimethionine methyl ester and similar compounds across the lysosomal membrane. Reeves (23) showed that leucine methyl ester is taken up rapidly by lysosomes and that it is hydrolyzed within lysosomes; free leucine was poorly transported, and similar findings were made with esters of phenylalanine, serine, and alanine. This approach was extended to other amino acids, notably cystine, by Steinherz *et al.* (24). These studies, which led to experiments in which leukocyte lysosomes were loaded with cystine by incubating the cells in media containing cystine dimethyl ester, suggest that the defect in cystinosis may be a deficiency of cystine export (25, 26).

The extent to which lysosomes may be involved in the uptake of GSH esters by liver and kidney remains to be explored. Additional work is also required on the uptake of GSH esters by other tissues (e.g., brain, muscle) and cell suspensions.

The present findings may have valuable experimental application. It seems reasonable to think that this approach will also provide a useful and probably safe way to increase the intracellular thiol levels of tissues. Various thiols have been proposed and used in clinical studies on radioprotection (27), but the toxicity of some of these compounds severely restricts their clinical application.

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