DECREASE IN GLUTATHIONE LEVELS OF KIDNEY AND LIVER AFTER INJECTION OF METHIONINE SULFOXIMINE INTO RATS

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After rats were injected with the convulsant methionine sulfoximine, there was a rapid decrease in the glutathione concentrations of the kidney and liver, but there was no measurable effect (within 5 hours) on brain glutathione. The maximum decreases in the glutathione concentrations of kidney and liver were observed 1 hr after injection and were about 60 and 40%, respectively, of the control levels. The findings suggest that there may be at least two pools of tissue glutathione. Studies in which other amino acids were injected, and earlier in vitro studies, are consistent with the conclusion that methionine sulfoximine affects glutathione synthesis in vivo by inhibiting γ-glutamylcysteine synthetase. Injection of glycylglycine also decreased glutathione levels, an effect probably mediated by γ-glutamyltranspeptidase.

Earlier work in this laboratory showed that the convulsant methionine sulfoximine inhibits glutamine synthetase by a mechanism in which one isomer of this amino acid (L-methionine-S-sulfoximine) is phosphorylated on the enzyme by ATP to yield L-methionine-S-sulfoximine phosphate, which binds tightly (but noncovalently) to the enzyme thus producing irreversible inhibition (1-4). Inhibition of glutamine synthetase by methionine sulfoximine was observed with enzyme preparations from brain (1-4), liver (5), and Escherichia coli (6), and also in in vivo studies in which the glutamine synthetase activity of brain, liver, and kidney of mice was found to be markedly reduced after injection of methionine sulfoximine (7,8).

Most of the previous studies on the action of methionine sulfoximine have dealt with effects on the brain and on inhibition of glutamine synthetase. Recent work in this laboratory on γ-glutamylcysteine synthetase (9) showed that this enzyme is also inhibited by L-methionine-S-sulfoximine in a manner similar to that found for glutamine

synthetase. It therefore seemed of interest, especially in relation to information recently developed about the γ-glutamyl cycle (a series of reactions which accounts for the synthesis and degradation of glutathione (GSH) (10-12)), to study the effect of injecting methionine sulfoximine on the tissue concentrations of GSH. In this work, substantial decreases in the concentrations of GSH in the kidney and liver were found after injection of methionine sulfoximine. Decreased GSH levels were also found after injection of glycylglycine, an effect which seems to reflect activity of another enzyme of the γ-glutamyl cycle, i.e., γ-glutamyltranspeptidase.

MATERIALS AND METHODS

L-Methionine-SR-sulfoximine, glycylglycine, L-methionine, monosodium L-glutamate, L-cysteine, L-α-aminobutyric acid, glutathione (GSH), glutathione disulfide (GSSG), methylglyoxal, 5,5'-dithiobis (2-nitrobenzoate) (DTNB), TPNH, yeast glyoxalase I and yeast glutathione reductase were obtained from Sigma.

Sprague-Dawley rats (280-300 g) were injected intraperitoneally with 2 ml of 0.9% NaCl (controls) or the same solution containing methionine sulfoximine (or other compounds); the animals were decapitated and the tissues were immediately excised and homogenized in 4 vols of 5% trichloroacetic acid at 4° with a Potter-Elvehjem homogenizer. After centrifugation at 10,000 x g for 20 min. at 4°, aliquots (1 ml) were extracted three times with 4 ml of ether to remove trichloroacetic acid, and the aqueous layers were analyzed for glutathione and "total glutathione" (GSH + GSSG). Methionine sulfoximine was determined on a Durrum Model D-500 amino acid analyzer. The values given here are the averages of determinations on tissue samples from 2-6 rats; the values obtained agreed with 10%.

GSH was determined by measuring S-lactoylglutathione formation from methylglyoxal in the presence of glyoxalase I (13). The assay mixture (1 ml) contained 100 μmoles of potassium phosphate (pH 6.6), 5 μmoles of methylglyoxal, and tissue extract.

The absorbance was measured at 240 nm before and 10 min. after addition of glyoxalase I (2.5 units); 25°. Under these conditions, S-lactoylglutathione exhibits a molar extinction coefficient of 3370. The recovery of GSH added to the samples was 96%. "Total glutathione" (GSH + GSSG) was determined (14) by spectrophotometric measurement of the catalytic effect of GSH and GSSG on the reduction of DTNB in the presence of glutathione reductase. The experimental and reference cuvettes contained assay mixtures (1 ml) consisting of 100 μmoles of potassium phosphate and 5 μmoles of EDTA (pH 7.5), 0.6 μmole of DTNB, 0.2 μmole of TPNH and 1.6 μg of glutathione reductase (0.2 unit). The reaction was started by adding the sample to the experimental cuvette and the rate of increase in absorbance at 412 nm at 25° was measured. Recoveries of added GSH and GSSG were 96%. Glutamine (15) and γ-glutamylcysteine (16) synthetases were determined as described.

RESULTS

After injection of methionine sulfoximine, the GSH concentrations of the kidney and liver decreased; the greatest effect was found about 1 hour after injection, and the values remained at about these levels for 5 hours (Fig. 1). The maximum decrease

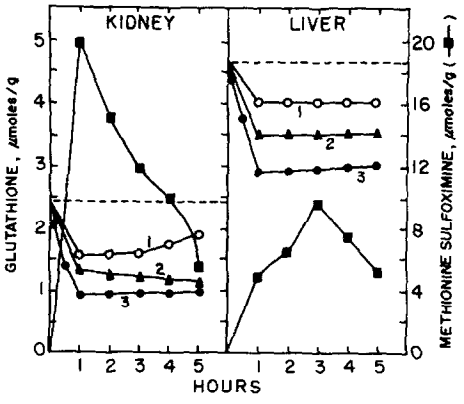

Fig. 1. Effect of intraperitoneal injection of methionine sulfoximine into rats on tissue GSH levels. Rats were given 0.92 (curves 1), 1.30 (curves 2), or 1.85 (curves 3) mmoles/Kg of L-methionine-SR-sulfoximine. Dotted lines indicate the control levels of GSH. Closed squares give values for tissue methionine sulfoximine concentration after injection of 1.85 mmoles/Kg of L-methionine-SR-sulfoximine.

was about the same in kidney and liver ($\sim$ 1.5 μmoles per g); however, the decrease on a percentage basis was greater in kidney ($\sim$ 60%) than in liver ($\sim$ 40%). Higher doses of methionine sulfoximine (up to 3.7 mmoles per Kg) produced convulsions and death within 1 hour but did not further decrease the GSH levels. No effect was observed on brain GSH under these conditions; values for brain GSH were 1.20-1.40 μmoles/g for both controls and treated animals. In all experiments both "total glutathione" and GSH were measured; the values agreed within experimental error indicating the presence of less than 5% of GSSG. The tissue methionine sulfoximine levels were determined after injection of 1.85 mmoles per Kg; the level in kidney rose to 20 μmoles/g after 1 hr and then decreased, while in liver the methionine sulfoximine level increased to about 10 μmoles/g and then declined (Fig. 1). In agreement with previous studies (7,8), no glutamine synthetase was detected in liver, kidney, or brain 1 hr after injection. γ-Glutamylcysteine synthetase activity in liver and kidney was found to be about 84% of the control; this result does not reflect in vivo enzymatic activity because, methionine sulfoximine phosphate is much less tightly bound to γ-glutamylcysteine synthetase than it is to glutamine synthetase, and under the conditions of assay, which involves considerable dilution, methionine sulfoximine phosphate is readily released from γ-glutamylcysteine synthetase (9). Injection of L-methionine, L-glutamate, L-cysteine, L-glutamine, and glycine did not affect GSH levels; L-α-aminobutyric acid produced a significant decrease in kidney GSH, and glycylglycine produced decreased kidney and liver GSH (Table 1).

DISCUSSION

That injection of methionine sulfoximine produced no decrease in the GSH level of brain under these conditions is consistent with previous findings that the overall turnover of GSH in brain is very slow compared to kidney and liver (17,18). It is unlikely that the substantial decrease in GSH levels of liver and kidney is due to increased

transpeptidation between GSH and methionine sulfoximine; although the latter is a substrate of γ-glutamyltranspeptidase (19), methionine and glutamine are better substrates and injection of these amino acids and also of cysteine, glutamate, and glycine did not affect GSH levels. However, the effect of glycylglycine, which is more active in transpeptidation than methionine (19), is probably due to depletion of GSH by transpeptidation. The effect of α-aminobutyric acid in lowering kidney GSH seems to reflect the ability of this amino acid to replace cysteine in the reaction catalyzed by γ-glutamylcysteine synthetase (20,21); α-aminobutyric acid and other amino acids that can replace cysteine are used for synthesis of the corresponding tripeptides in vivo (22,23). These would not be detected by the assays used here, which require the SH-group of GSH. The marked decrease of the GSH levels of kidney and liver after injection of methionine sulfoximine is most likely due to inhibition of γ-glutamylcysteine synthetase, but other explanations cannot be unequivocally excluded. The γ-glutamylcysteine synthetase activity per g of rat kidney is about 20 times that of liver (16); in contrast, the activity of γ-glutamylcysteine synthetase in brain is much lower. The results suggest that the convulsant action of methionine sulfoximine is not mediated directly through a mechanism involving glutathione, but the possibility exists that methionine sulfoximine may decrease the GSH levels in specific regions or cells of the brain in which GSH turnover is very high. In this regard it is of interest that the GSH levels in kidney and liver did not decrease more than about 60% in kidney and about 40% in liver; further decreases did not occur even when much larger doses of methionine sulfoximine were given. The tissue levels of methionine sulfoximine achieved here would be expected to inhibit the enzyme almost completely. The findings suggest that there may be at least 2 pools of GSH and that inhibition of glutathione synthesis by methionine sulfoximine affects a pool that turns over relatively rapidly. It is also conceivable that some of the enzyme is not accessible to the injected methionine sulfoximine. Although several interesting questions remain to

TABLE I

Effect of Injecting Various Compounds on Levels of GSH in Kidney and Liver

Compound Injected	μmoles of GSH/g*	
	Kidney	Liver
None	2.44	4.66
L-Methionine-SR-sulfoximine (1.85)+	0.95	2.90
L-Methionine (1.85)	2.10	4.70
L-Glutamate (1.67)	2.10	4.28
L-Cysteine (1.67)	2.17	4.90
L-Glutamine (1.67)	2.50	
Glycine (3.34)	2.22	4.50
L-α-Aminobutyrate (1.67)	1.59	4.30
L-α-Aminobutyrate (6.66)	1.24	4.15
Glycylglycine (1.67)	1.86	4.55
Glycylglycine (3.34)	1.23	4.12
Glycyl-L-valine (1.67)	1.95	
L-Alanyl-glycine (1.67)	2.18	
L-Alanyl-L-alanine (1.67)	2.18	

*Values determined 1 hr. after injection. + Figures in parentheses indicate dose given (mmoles/Kg).

be answered about the effect of methionine sulfoximine on tissue levels of GSH, the present findings suggest that methionine sulfoximine may prove useful as an inhibitor of GSH synthesis in various experimental systems.

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