Characterization of the enzymic capacity for cysteine desulphhydration in liver and kidney of the rat

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The contribution of cystathionine γ -lyase, cystathionine β -synthase and cysteine aminotransferase coupled to 3-mercaptopyruvate sulphurtransferase to cysteine desulphhydration in rat liver and kidney was assessed with four different assay systems. Cystathionine γ -lyase and cystathionine β -synthase were active when homogenates were incubated with 280 mM-L-cysteine and 3 mM-pyridoxal 5'-phosphate at pH 7.8. Cysteine aminotransferase in combination with 3-mercaptopyruvate sulphurtransferase catalysed essentially all of the H_2S production from cysteine at pH 9.7 with 160 mM-L-cysteine, 2 mM-pyridoxal 5'-phosphate, 3 mM-2-oxoglutarate and 3 mM-dithiothreitol. At more-physiological concentrations of cysteine (2 mM) cystathionine γ -lyase and cystathionine β -synthase both appeared to be active in cysteine desulphhydration, whereas the aminotransferase pathway did not. The effect of inhibition of cystathionine γ -lyase by a suicide inactivator, propargylglycine, in the intact rat was also investigated; there was no significant effect of propargylglycine administration on the urinary excretion of total ^{35}S , $^{35}\text{SO}_4^{2-}$ or $[^{35}\text{S}]$ taurine formed from labelled dietary cysteine.

The desulphhydration of cyst(e)ine may be catalysed by several enzymes present in mammalian tissues. These include cysteine aminotransferase (EC 2.6.1.3) in conjunction with 3-mercaptopyruvate sulphurtransferase (EC 2.8.1.2), cystathionine γ -lyase (EC 4.4.1.1) and cystathionine β -synthase (EC 4.2.1.22). The reactions catalysed by these enzymes are summarized in Scheme 1. The reactions may be important not only in cysteine catabolism but also as a source of metabolically active reduced sulphur. Other known pathways of cysteine degradation in animal tissues lead to release of sulphur in the 4+ or 6+ oxidation state. This highly oxidized sulphur is irreversibly lost to the pool of metabolically active reduced sulphur because of the absence of sulphate- and sulphite-reducing systems in animal tissues (Greenberg, 1975).

Cysteine participates in transamination reactions with various amino acceptors, and it is likely that several non-specific aminotransferases catalyse its transamination to 3-mercaptopyruvate (Meister *et al.*, 1954; Ip *et al.*, 1977; Ubuka *et al.*, 1977*a,b*, 1978). Aspartate aminotransferase catalyses the transamination of cysteine with 2-oxoglutarate *in vitro* and may also be active *in vivo* (Taniguchi & Kimura, 1974). The keto acid of cysteine is decomposed to inorganic sulphur and pyruvate by both desulphuration and trans-sulphuration reactions (Meister *et al.*,

1954; Kun & Fanshier, 1958, 1959*a,b*). These reactions are catalysed by the same enzyme, 3-mercaptopyruvate sulphurtransferase, which apparently has an absolute specificity for 3-mercaptopyruvate as the sulphur donor (Fiedler & Wood, 1956). The sulphurtransferase can transfer the sulphur from 3-mercaptopyruvate to sulphite to form thiosulphate or to other sulphur acceptors such as thiol compounds, sulphinates and cyanide (Meister *et al.*, 1954; Kun & Fanshier, 1958, 1959*a,b*). When no sulphur-accepting substrate is present the atomic sulphur of the enzyme-sulphur complex is apparently released and may be polymerized to form elemental sulphur, reduced to sulphide or oxidized to sulphate (Meister *et al.*, 1954; Kun & Fanshier, 1959*a*). Free H_2S may be formed by reduction of the atomic sulphur or released from products such as thiosulphate and persulphides in subsequent reactions, as summarized in reactions 1A–1C of Scheme 1. H_2S may be released from thiosulphate, as illustrated in the inset in Scheme 1, in a reaction catalysed by thiosulphate sulphurtransferase (EC 2.8.1.1) or a glutathione-dependent thiosulphate reductase (Koj *et al.*, 1967; Koj, 1968; Uhteg & Westley, 1979).

Cystathionine γ -lyase catalyses the desulphhydration of certain disulphides, including cystine (Cavallini *et al.*, 1962*a,b*; Szczepkowski & Wood,

1967). Cystine is believed to undergo cystathionine γ -lyase-catalysed desulphhydration by the scheme summarized in reactions 2+2A–2C of Scheme 1. Cystathionine γ -lyase catalyses a β -disulphide elimination reaction that results in the production of pyruvate, NH_4^+ and thiocysteine (reaction 2). Thiocysteine may react with cysteine or other thiols to form H_2S and cystine or the corresponding disulphide (reaction 2A). Thiocysteine may also decompose to elemental sulphur and cysteine (reaction 2B), or the sulphur may be transferred from thiocysteine to acceptors such as sulphite or sulphinates (reaction 2C).

Cystathionine β -synthase can catalyse a wide range of β -replacement reactions, which include the substitution of the thiol group of cysteine with a variety of thiol compounds to form H_2S and the corresponding thioether, as illustrated in reaction 3 of Scheme 1 (Lak *et al.*, 1970; Braunstein *et al.*, 1971; Porter *et al.*, 1974).

The aims of the work reported in the present paper were: (1) to assess the contribution of each of the three pathways of cysteine desulphhydration under both optimal and more-physiological assay conditions; (2) to determine the effect of administration of propargylglycine [2-aminopent-4-ynoic acid, a suicide inhibitor of cystathionine γ -lyase (Abeles & Walsh, 1973; Washtien *et al.*, 1977)] on the excre-

tion of cysteine sulphur as sulphate and taurine by the intact rat.

Methods and materials

Animals and diet

Male rats of the Sprague–Dawley strain (Blue Spruce Farms, Altamont, NY, U.S.A.) that weighed between 93 and 320 g were used for all experiments. Rats were housed in individual suspended stainless-steel cages in a room maintained at 23°C and with a 12h-light/12h-dark cycle. The dark period was between 10:00 and 22:00h. All animals had access *ad libitum* to water and a modification of the AIN-76A diet (AIN Ad Hoc Committee on Standards for Nutritional Studies, 1977, 1980) that contained (% by wt.) 20% vitamin-free casein (ICN Pharmaceuticals, Cleveland, OH, U.S.A.), 32.5% sucrose, 32.5% corn starch, 5% cellulose (Alphacel; ICN Pharmaceuticals), 5% corn oil (Mazola; CPC International, Englewood Cliffs, NJ, U.S.A.), 1% AIN-76A vitamin mix and 3.5% AIN-76 mineral mix (ICN Pharmaceuticals), 0.2% choline bitartrate and 0.3% L-methionine. Rats were maintained under these conditions for at least 1 week before experimental treatments were begun.

Assays of enzyme activity

The formation of H_2S from cysteine by rat liver

Scheme 1. Pathways of H_2S production from cysteine in mammalian tissues

Reaction 1 is catalysed by cysteine aminotransferase, reactions 1A–1C by 3-mercaptopyruvate sulphydratase, reaction 2 by cystathionine γ -lyase, and reaction 3 by cystathionine β -synthase. CySH, cysteine; RSH, thiol; RSSH, persulphide; RSSR, disulphide.

and kidney homogenates was measured with four different assay systems, referred to below as cysteine desulphhydration assays I, II, III and IV. All determinations were made in triplicate. The incubation mixtures for assays I and II were based on preliminary investigations of varied substrate and cofactor concentrations and pH to establish the requirements for maximal H_2S formation by both rat liver and kidney homogenates. A linear dependence of activity on tissue concentration and incubation time was observed with both assay systems. Assay II incubation mixture included a keto acid (2-oxoglutarate), which is required for the aminotransferase pathway of H_2S production, and dithiothreitol, which was added to minimize oxidation of cysteine to cystine. 2-Oxoglutarate and dithiothreitol were omitted from assay I incubation mixture, which contained 280 mM-L-cysteine (free base), 3 mM-pyridoxal 5'-phosphate, 100 mM-Tris/HCl buffer, pH 7.8, and 0.3 ml of a 4% (w/v) liver homogenate or 10% (w/v) kidney homogenate in 50 mM-potassium phosphate buffer, pH 6.8, in a final volume of 1.0 ml; the pH of the complete incubation mixture was adjusted to 7.8. The incubation mixture for assay II contained 160 mM-L-cysteine, 2 mM-pyridoxal 5'-phosphate, 3 mM-dithiothreitol, 3 mM-2-oxoglutarate, 100 mM-Tris/HCl buffer, pH 9.7, and 0.3 ml of a 1% (w/v) liver or kidney homogenate in 50 mM-potassium phosphate buffer, pH 6.8, in a final volume of 1.0 ml; the pH of the complete incubation mixture was adjusted to 9.7. When homogenates of brain, heart and gastrocnemius muscle were used for these two assays, 40% (w/v) homogenates were used for assay I and 0.5%, 1% and 4% (w/v) homogenates of heart, skeletal muscle and brain respectively were used for assay II.

The incubation conditions for cysteine desulphhydration assays III and IV were fixed at 2 mM-cysteine and pH 7.4 to be closer to physiological conditions. Concentrations of pyridoxal 5'-phosphate, 2-oxoglutarate and dithiothreitol were those that yielded maximal activity in rat liver and kidney homogenates. A linear dependence of activity on tissue concentration and incubation time was observed. Assay III incubation mixture contained, in a final volume of 1.0 ml, 2 mM-cysteine, 0.05 mM-pyridoxal 5'-phosphate, 100 mM-potassium phosphate buffer, pH 7.4, and 0.3 ml of 40% (w/v) liver or 30% (w/v) kidney homogenate in 50 mM-potassium phosphate buffer, pH 6.8; the pH of the complete incubation mixture was adjusted to 7.4. The incubation mixture for assay IV was the same as that for assay III except that 0.5 mM-2-oxoglutarate and 0.05 mM-dithiothreitol were included.

All incubations were performed in 25 ml Erlenmeyer flasks fitted with septum stoppers and plastic centre wells (Kontes, Vineland, NJ, U.S.A.). All components of the incubation mixtures and the

tissue homogenates were added to the flasks while the flasks were in an ice bath. Centre wells were filled with 0.5 ml of a 15:1 (v/v) mixture of 1% (w/v) zinc acetate and 12% (w/v) NaOH and a folded 2 cm $\times$ 2.5 cm rectangle of Whatman no. 1 filter paper for trapping evolved H_2S (Fogo & Popowsky, 1949). Each flask was flushed with N_2 for 20 s and then sealed.

The reactions were initiated by transferring the flasks to a 37°C shaking water bath at 20 s intervals. After 15 min at 37°C, reactions were stopped by injection of 0.5 ml of 50% (w/v) trichloroacetic acid through the diaphragm of each septum stopper. Flasks were left in the shaking water bath at 37°C for 1 h after the reactions were stopped to allow complete trapping of H_2S . Blank incubations were performed for all samples in a similar manner except that the tissue preparations were first heated at 100°C for 2 min.

H_2S was measured as described by Siegel (1965). The centre wells and contents were transferred to test tubes, and 3.5 ml of water was added to each tube. To each tube, 0.4 ml of 20 mM-*NN*-dimethyl-*p*-phenylenediamine sulphate in 7.2 M-HCl was added. This was followed immediately by addition of 0.4 ml of 30 mM- FeCl_3 in 1.2 M-HCl. After 10 min, the absorbance of the resulting solution at 670 nm was determined with a Gilford Stasar III spectrophotometer (Gilford Instrument Laboratories, Oberlin, OH, U.S.A.). The ϵ_{670} for sulphide was $3.38 \times 10^4 \text{ M}^{-1} \cdot \text{cm}^{-1}$; standard sulphide solutions were calibrated by reaction of the sulphide with iodine and titration of the excess iodine with standard thiosulphate (Roy & Trudinger, 1970).

Cystathionine γ -lyase, cysteine:2-oxoglutarate aminotransferase and cystathionine β -synthase activities were determined as previously described (Stipanuk & Benevenga, 1977; Stipanuk, 1979). The products and reactions monitored were the formation of 2-oxobutyrates by cleavage of cystathionine, the formation of glutamate by transamination of cysteine and 2-oxoglutarate and the synthesis of cystathionine from serine and homocysteine.

The protein concentration of tissue preparations was determined by the method of Lowry *et al.*, (1951). Bovine serum albumin was used as the standard.

All rats were killed by decapitation between 09:00 and 12:00 h. The liver, both kidneys, heart, brain and the right gastrocnemius muscle were immediately removed, rinsed with ice-cold 0.9% NaCl, placed on ice and then homogenized in 4 vol. of 50 mM-potassium phosphate buffer, pH 6.8, with a Polytron homogenizer (Brinkmann Instruments, Westbury, NY, U.S.A.). The 20% (w/v) homogenates were subsequently diluted with additional homogenization buffer to yield the appropriate tissue concentration.

DL-Propargylglycine (Sigma Chemical Co., St. Louis, MO, U.S.A.) was added to some tissue homogenates, which were then incubated at 37°C for 5 min before measurement of enzyme activity; the amount of propargylglycine added to each tissue preparation was calculated to yield 2 mM-propargylglycine when the tissue preparation was diluted in the complete incubation mixture. *S*-Adenosyl-L-methionine (chloride salt; Sigma) was added to tissue homogenates to yield a concentration of 2 mM in 20% (w/v) tissue homogenates; these homogenates were incubated at 37°C for 15 min before measurements of enzyme activity were made. Control determinations were made on untreated tissue preparations, which were from the same rats and had been incubated in the same manner as the treated tissue preparations.

A group of six rats was given 30 μ mol of DL-propargylglycine per 100 g body wt. by intraperitoneal injection of a 60 mM solution of DL-propargylglycine in 0.9% NaCl 3 h before the rats were killed. A second group of five rats was given 236 μ mol of L-ethionine (Sigma) per 100 g body wt. by intraperitoneal injection of a solution of 154 mM-L-ethionine in 0.9% NaCl 2.5 h before the rats were killed. Two other groups of rats with similar body weights were injected with the corresponding volumes of saline and used as control groups. Rats were randomly assigned to treatment and control groups. Injections were made at 09:00 h.

Effect of propargylglycine on the 24 h urinary excretion of cysteine sulphur as sulphate and taurine

Twenty-four rats that weighed between 122 and 162 g were randomly distributed among six treatment groups. Two of these groups were fed *ad libitum* for 2 days on an L-amino acid diet that was the same as the 20%-casein diet except that the casein was replaced by 16.94% of an L-amino acid mixture, the amounts of sucrose and corn starch were each increased to 34.18% and the diet contained 6 mmol of DL-propargylglycine per kg of diet. The L-amino acid mixture was a modification of that of Rogers & Harper (1965) and contained (% of diet, by wt.) 1.12% arginine, 0.30% histidine, 0.80% isoleucine, 1.08% leucine, 1.76% lysine hydrochloride, 0.40% methionine, 0.60% cysteine, 1.14% phenylalanine, 0.17% tryptophan, 0.80% valine, 0.34% alanine, 0.34% aspartic acid, 3.41% glutamic acid, 2.28% glycine, 0.34% proline, 0.34% serine, 0.34% tyrosine, 0.60% asparagine and 0.79% sodium acetate. The amino acid diet was used because a diet in which the cysteine content could be varied and in which cysteine was present in free form was needed for the tracer study on day 3. The other four groups of rats were fed on a control diet that contained no propargylglycine; the rats in two of these groups were fed *ad libitum* and those in the

remaining two groups were pair-fed to the rats given propargylglycine. Food was placed in the cages just before the beginning of the dark cycle each day. Food intake and body weight of the rats were measured daily.

Between 09:00 and 10:00 h on the third day, 5 g of the L-amino acid diet mixed with 4 ml of distilled water and labelled with 2 μ Ci of L-[³⁵S]cysteine (Amersham Corp., Arlington Heights, IL, U.S.A.) was administered to each rat by gastric intubation. The diet given to each rat was the same as that eaten on the previous 2 days except that the L-cysteine (free base) content of the diet was 0.2% or 2.6% rather than 0.6%. The preparation and administration of the liquid diets and the determination of the exact amount of L-[³⁵S]cysteine administered to each rat were performed as previously described (Stipanuk, 1979).

Rats were placed in individual metabolic cages immediately after they received the labelled diets, and urine was collected for 24 h in vials that contained 5 ml of 2% (w/v) phenol. Rats had access to water but not to food during this 24 h period. Rats were killed at the end of the 24 h period. The bladder contents of each rat were added to the urine collections, and the 'unabsorbed' radioactivity in the gastrointestinal tracts of the rats was determined as previously described (Stipanuk & Benevenga, 1977; Stipanuk, 1979). The amounts of total ³⁵S radioactivity, [³⁵S]taurine and ³⁵SO₄²⁻ in the 24 h urine collections were determined by methods previously described (Stipanuk, 1979). Results are reported as percentages of the absorbed (administered minus unabsorbed) dose of L-[³⁵S]cysteine.

To ascertain that cystathionine γ -lyase activity was inhibited in tissues of the rats that were given propargylglycine, 18 additional rats (104–129 g body wt.) were randomly assigned to the same six treatment or control groups and fed on the same L-amino acid diets for 2 days. On the third day at 08:00 h, these rats were given the same L-amino acid diets by gastric intubation except that the liquid diets were not labelled with L-[³⁵S]cysteine. At 8 h after intubation of the liquid diets, the rats were killed by decapitation and liver and kidneys were removed. The cystathionine γ -lyase activity and the rate of H₂S production from cysteine (desulphhydration assay system I) were determined for liver and kidney.

Statistics

Data were analysed by using either Student's *t* test or paired *t* test [Minitab-80.1 (Pennsylvania State University, University Park, PA, U.S.A.) employed with the Cornell University Computer System]. Levels of significance have been reported when *P* < 0.10.

Results

Tissue distribution of capacity for H₂S production

The distribution of the capacity for H₂S production in rat tissues was determined with assay systems I and II, which yielded maximal rates of H₂S formation in the absence (I) and in the presence (II) of 2-oxoglutarate and dithiothreitol. As shown in Table 1, H₂S production in assay system II was much higher than that in system I in all tissues. H₂S production in assay system I was greatest in liver and kidney, which corresponds to the distribution of cystathionine γ -lyase and cystathionine β -synthase activities in rat tissues (Tallan *et al.*, 1958; Mudd *et al.*, 1965; Sturman *et al.*, 1970; Finkelstein *et al.*, 1971). H₂S production in assay system II was greatest in heart, liver and kidney; this activity distribution is similar to that observed for cysteine:2-oxoglutarate aminotransferase (Ishimoto, 1979; S.-M. Kuo, T. C. Lea & M. H. Stipanuk, unpublished work), as well as aspartate aminotransferase (Herzfeld & Greengard, 1971), which can catalyse the transamination of cysteine (Taniguchi & Kimura, 1974). The pH value and substrate and cofactor concentrations that yielded maximal rates of H₂S production from cysteine in rat liver with assay II were almost the same as those found to give maximal and similar rates of glutamate production in the transamination of cysteine with 2-oxoglutarate (S.-M. Kuo, T. C. Lea & M. H. Stipanuk, unpublished work). Thus assay system II appears to measure primarily, if not entirely, the combined activity of cysteine aminotransferase and 3-mercaptopyruvate sulphurtransferase.

Influence of pyridoxal 5'-phosphate and 2-oxoglutarate on H₂S production

As shown in Table 2 for assay system I, which yielded maximal activity for rat liver and kidney homogenates in the absence of 2-oxoglutarate, the omission of pyridoxal 5'-phosphate resulted in a

78% and a 45% decrease in H₂S production in liver and kidney respectively. On the other hand, omission of pyridoxal 5'-phosphate from assay system II, which yielded a maximal rate of H₂S production for rat liver and kidney homogenates in the presence of 2-oxoglutarate, had no effect on H₂S production by either liver or kidney. Omission of pyridoxal 5'-phosphate from the more-physiological assay systems, III and IV, resulted in a 22% and a 43% decrease for liver and a 35% and a 29% decrease for kidney in H₂S production in assay systems III and IV respectively. Thus exogenous pyridoxal 5'-phosphate appeared to be essential for maximal activity in assay systems I, III and IV but not in assay system II. The similar requirement for pyridoxal 5'-phosphate in assay systems I, III and IV suggests that the same enzyme is involved in catalysis of H₂S production in the three systems and that a different enzyme is involved in assay system II.

Addition of 2-oxoglutarate increased H₂S production in assay system I by 256% and 369% in liver and kidney respectively, whereas omission of 2-oxoglutarate from assay system II resulted in a 92% and a 96% decrease in activity in liver and kidney respectively. Addition of 2-oxoglutarate to assay system III had no significant effect on H₂S production by either liver or kidney; omission of 2-oxoglutarate from assay system IV similarly had no effect on H₂S production by liver but decreased H₂S production by kidney by 21%. These observations suggest either that cysteine transamination did not play a large role in cysteine desulphhydration under the more-physiological incubation conditions used in assay systems III and IV or that endogenous keto acids in the tissue preparations were adequate for cysteine transamination in these assay systems. The near-absolute dependency of activity in assay system II on the addition of 2-oxoglutarate supports the previous conclusion that this assay system primarily is a measure of the combined activities of cysteine aminotransferase and mercaptopyruvate sulphurtransferase.

The influence of dithiothreitol on the rate of cysteine desulphhydration in each of the four assay systems was also investigated. Because cystathionine γ -lyase uses cystine rather than cysteine as its substrate, we added dithiothreitol to minimize formation of cystine, and hence the desulphhydration of cystine by cystathionine γ -lyase. Although addition of 3 mM-dithiothreitol to assay system II resulted in a small and consistent increase in H₂S production, no statistically significant effect of dithiothreitol was observed in any of the assay systems. It seems likely that dithiothreitol has non-specific effects that may enhance the production of H₂S by some pathways and decrease the production of H₂S by other pathways.

Table 1. *Distribution of enzymic capacity for cysteine desulphhydration in rat tissues*

Experimental details are given in the text. Values are means $\pm$ S.D. for four or five rats whose body weights ranged from 190 to 269 g.

Tissue	Cysteine desulphhydration (μ mol of H ₂ S/min per g)	
	Assay I	Assay II
Liver	0.645 $\pm$ 0.082	3.78 $\pm$ 0.52
Kidney	0.250 $\pm$ 0.072	2.26 $\pm$ 0.15
Heart	0.037 $\pm$ 0.019	4.11 $\pm$ 0.14
Brain	0.018 $\pm$ 0.010	1.42 $\pm$ 0.09
Skeletal muscle	0.010 $\pm$ 0.004	1.67 $\pm$ 0.10

Table 2. *Effect of addition or omission of assay components on the production of H₂S by rat liver and kidney homogenates*. Experimental details are given in the text. Values for control activities are means $\pm$ S.D. for 11 rats. Values expressed as percentages of control are means $\pm$ S.D. for three to seven rats. The level of significance was determined with the paired *t* test.

Cysteine desulphhydration assay system	Control activity (μ mol of H ₂ S/min per g)		Addition or omission	H ₂ S production (% of control)	
	Liver	Kidney		Liver	Kidney
I: 280 mM-L-cysteine, 3 mM-pyridoxal 5'-phosphate, pH 7.8	0.403 $\pm$ 0.154	0.192 $\pm$ 0.065	–Pyridoxal 5'-phosphate	22 $\pm$ 7 <i>P</i> < 0.003	55 $\pm$ 7 <i>P</i> < 0.008
			+2-Oxoglutarate (3 mM)	356 $\pm$ 21 <i>P</i> < 0.0001	469 $\pm$ 72 <i>P</i> < 0.002
II: 160 mM-L-cysteine, 2 mM-pyridoxal 5'-phosphate, 3 mM-2-oxoglutarate, 3 mM-dithiothreitol, pH 9.7	2.31 $\pm$ 0.57	1.96 $\pm$ 0.44	–Pyridoxal 5'-phosphate	100 $\pm$ 8	96 $\pm$ 11
			–2-Oxoglutarate	8 $\pm$ 4 <i>P</i> < 0.0001	4 $\pm$ 4 <i>P</i> < 0.0001
III: 2 mM-L-cysteine, 0.05 mM-pyridoxal 5'-phosphate, pH 7.4	0.017 $\pm$ 0.003	0.013 $\pm$ 0.001	–Pyridoxal 5'-phosphate	78 $\pm$ 15 <i>P</i> < 0.007	64 $\pm$ 6 <i>P</i> < 0.0001
			+2-Oxoglutarate (0.5 mM)	96 $\pm$ 29	137 $\pm$ 37
IV: 2 mM-L-cysteine, 0.05 mM-pyridoxal 5'-phosphate, 0.5 mM-2-oxoglutarate, 0.05 mM-dithiothreitol, pH 7.4	0.019 $\pm$ 0.006	0.018 $\pm$ 0.003	–Pyridoxal 5'-phosphate	57 $\pm$ 9 <i>P</i> < 0.0001	71 $\pm$ 9 <i>P</i> < 0.0001
			–2-Oxoglutarate	98 $\pm$ 15	79 $\pm$ 8 <i>P</i> < 0.04

Effects of ethionine and S-adenosylmethionine on the activities of enzymes involved in H₂S production

We used two approaches in an effort to enhance cystathionine β -synthase activity selectively. Ethionine (246 μ mol/100 g body wt.) or saline was administered to intact rats, which were killed 2.5 h later for determination of enzyme activities in liver and kidney of these rats. The second approach was to incubate homogenates of liver and kidney from untreated rats with *S*-adenosylmethionine before the determination of enzyme activities.

Administration of ethionine to the intact rat did not markedly increase hepatic cystathionine β -synthase activity, as had been reported by Koracevic and co-workers (Koracevic, 1975; Koracevic *et al.*, 1979) and Finkelstein *et al.* (1975). Only a 33% increase (*P* < 0.07) in the activity of hepatic cystathionine β -synthase was observed. A 16% increase (*P* < 0.07) in the rate of cysteine desulphhydration by rat liver in assay system I was also observed. No increase in either activity was observed for kidney. The increases in both cystathionine β -synthase activity and H₂S production from cysteine in assay system I that were observed for liver, although small, are consistent with involvement of cystathionine β -synthase in the catalysis of cysteine desulphhydration by rat liver.

Compared with the treatment of intact rats with ethionine, incubation of liver and kidney homogenates with *S*-adenosylmethionine had similar but somewhat greater effects. As shown in Table 3, the

activity of cystathionine β -synthase was increased in liver by 44% and in kidney by 27%, but these increases were not statistically significant. The 44% increase in hepatic cystathionine β -synthase activity is smaller than the 166% increase reported by Finkelstein *et al.* (1975) but similar to the response that we observed to treatment of intact rats with ethionine. Pretreatment of homogenates with *S*-adenosylmethionine increased H₂S production from cysteine as measured in assay system I by 39% in liver and 56% in kidney, but it had no effect on H₂S production as measured in assay system II, which primarily measures cysteine aminotransferase plus mercaptopyruvate sulphurtransferase activities. *S*-Adenosylmethionine was also effective in increasing H₂S production from cysteine in both of the more-physiological assay systems; increases of 47% and 42% with assay system III and of 75% and 20% with assay system IV were seen for liver and kidney respectively. No effect of *S*-adenosylmethionine on cystathionine γ -lyase or cysteine aminotransferase activity was observed.

Effect of propargylglycine on the activities of enzymes involved in H₂S production

Abeles and co-workers (Abeles & Walsh, 1973; Washtien *et al.*, 1977) have reported the irreversible inactivation of cystathionine γ -lyase by low concentrations (60 μ M) of propargylglycine. Uren *et al.* (1978) observed cystathioninaemia and cystathioninuria in mice treated for 4 days with daily injec-

tions of 12 μ mol of DL-propargylglycine per 100 g body wt. DL-Propargylglycine was used in the present study as a specific covalent inhibitor of cysteine desulphhydration catalysed by cystathionine γ -lyase. Data in Table 4 show that incubation of tissue preparations with propargylglycine inhibited cystathionine γ -lyase activity by 93% in both liver and kidney; propargylglycine did not significantly affect cysteine:2-oxoglutarate aminotransferase activity in liver or kidney or cystathionine β -synthase activity in liver. Cystathionine β -synthase activity in kidney homogenates was slightly (13%) lower in treated than in control preparations.

Incubation of liver and kidney homogenates with propargylglycine before the assay decreased H_2S production with assay system I by 87% and 74% in liver and kidney respectively, but had no signi-

ficant effect with assay system II. In the more-physiological assays of cysteine desulphhydration, pretreatment with propargylglycine decreased H_2S production by liver and kidney homogenates by 68% and 30% respectively with assay system III and by 51% and 32% respectively with assay system IV.

These results indicate that cystathionine γ -lyase catalysed little or none of the observed desulphhydration of cysteine in assay system II, whereas it was responsible for most of the desulphhydration of cysteine in assay system I. The residual activity in assay system I in the presence of propargylglycine may have been due to H_2S production catalysed by other enzymes (cystathionine β -synthase or cysteine aminotransferase plus mercaptopyruvate sulphurtransferase) or to a small amount of cyst-

Table 3. *Effect of incubation of rat liver and kidney homogenates with S-adenosyl-L-methionine on the activities of enzymes that catalyse the production of H_2S from cysteine*

Experimental details are given in the text. Values are means $\pm$ S.D. for four rats. The level of significance was determined by the paired *t* test.

Enzyme activity (product measured)	Activity (% of control)	
	Liver	Kidney
Cysteine desulphhydration assay I (H_2S)	139 $\pm$ 10 <i>P</i> < 0.005	156 $\pm$ 26 <i>P</i> < 0.02
Cysteine desulphhydration assay II (H_2S)	104 $\pm$ 4	102 $\pm$ 3
Cysteine desulphhydration assay III (H_2S)	147 $\pm$ 32 <i>P</i> < 0.06	142 $\pm$ 26 <i>P</i> < 0.05
Cysteine desulphhydration assay IV (H_2S)	175 $\pm$ 46 <i>P</i> < 0.05	120 $\pm$ 11 <i>P</i> < 0.04
Cystathionine γ -lyase (2-oxobutyrate)	106 $\pm$ 6	101 $\pm$ 4
Cystathionine β -synthase (cystathionine)	144 $\pm$ 29 <i>P</i> < 0.01	127 $\pm$ 40
Cysteine:2-oxoglutarate aminotransferase (glutamate)	97 $\pm$ 2	109 $\pm$ 9

Table 4. *Effect of incubation of rat liver and kidney preparations with DL-propargylglycine on the activities of enzymes that catalyse the production of H_2S from cysteine*

Experimental details are given in the text. Values are means $\pm$ S.D. for four to six rats. The level of significance was determined by the paired *t* test.

Enzyme activity (product measured)	Activity (% of control)	
	Liver	Kidney
Cysteine desulphhydration assay I (H_2S)	13 $\pm$ 5 <i>P</i> < 0.0001	26 $\pm$ 8 <i>P</i> < 0.0001
Cysteine desulphhydration assay II (H_2S)	98 $\pm$ 12	102 $\pm$ 5
Cysteine desulphhydration assay III (H_2S)	32 $\pm$ 11 <i>P</i> < 0.0001	70 $\pm$ 15 <i>P</i> < 0.004
Cysteine desulphhydration assay IV (H_2S)	49 $\pm$ 22 <i>P</i> < 0.002	68 $\pm$ 20 <i>P</i> < 0.01
Cystathionine γ -lyase (2-oxobutyrate)	7 $\pm$ 7 <i>P</i> < 0.0001	7 $\pm$ 6 <i>P</i> < 0.0001
Cystathionine β -synthase (cystathionine)	95 $\pm$ 14	87 $\pm$ 8 <i>P</i> < 0.04
Cysteine:2-oxoglutarate aminotransferase (glutamate)	103 $\pm$ 6	106 $\pm$ 4

athionine γ -lyase activity that was not inhibited by propargylglycine.

The effect of an intraperitoneal injection of DL-propargylglycine (30 μ mol per 100 g body wt.) 3 h before the rats were killed is summarized in Table 5. Results were similar to those shown in Table 4 for the pretreatment of tissue preparations with propargylglycine. Cystathionine γ -lyase activity was nearly completely inhibited in both liver and kidney (95% and 96%), and cysteine desulphhydration in assay system II was decreased by 67% in liver and 50% in kidney of rats that had been injected with propargylglycine.

Effect of propargylglycine on L-[35 S]cysteine metabolism in the intact rat

Because propargylglycine is effective in inhibiting cystathionine γ -lyase activity in the intact rat (Table 5; Stipanuk, 1977; Uren *et al.*, 1978), we investigated the effect of feeding with propargylglycine (6 mmol/kg of diet) for 3 days on the 24 h urinary excretion of sulphate and taurine from labelled dietary

cysteine. We hypothesized that we might see a decrease in urinary SO_4^{2-} excretion relative to taurine excretion if cystathionine γ -lyase-catalysed desulphhydration of cysteine plays a major role in cysteine degradation in the intact rat. The results are reported in Table 6. Two concentrations of cysteine were included in the labelled diets that were intubated on the third day, a typical dietary content, 0.2%, and an excess dose, 2.6%. Whereas the control rats fed *ad libitum* gained weight, rats given propargylglycine consumed less diet than did the control rats and lost weight. The food intake and body-weight change of the pair-fed control groups were intermediate between those of the control groups fed *ad libitum* and the groups given propargylglycine. Because rats were pair-fed for only 2 days and intake for the first day was estimated from a preliminary study, there was some difference between the mean total food intake of the rats given propargylglycine and that of their pair-fed controls.

The excretion of total ^{35}S and $^{35}\text{SO}_4^{2-}$ was similar for rats treated with propargylglycine and both the

Table 5. *Effect of injection of DL-propargylglycine on the activities of enzymes that catalyse the production of H_2S from cysteine in rat liver and kidney*

Experimental details are given in the text. Values are means $\pm$ S.D. for five or six rats. Values followed by an asterisk (*) are significantly different from the control value at $P < 0.0001$ by Student's *t* test.

Enzyme activity (product measured)	Activity (μ mol/min per g)			
	Liver		Kidney	
	Control	+Propargylglycine	Control	+Propargylglycine
Cysteine desulphhydration assay I (H_2S)	0.36 $\pm$ 0.03	0.15 $\pm$ 0.03*	0.19 $\pm$ 0.03	0.09 $\pm$ 0.01*
Cysteine desulphhydration assay II (H_2S)	3.1 $\pm$ 0.3	3.0 $\pm$ 0.3	2.0 $\pm$ 0.3	1.7 $\pm$ 0.2
Cystathionine γ -lyase (2-oxobutyrates)	0.80 $\pm$ 0.26	0.04 $\pm$ 0.02*	0.24 $\pm$ 0.07	0.02 $\pm$ 0.02*
Cystathionine β -synthase (cystathionine)	0.10 $\pm$ 0.02	0.12 $\pm$ 0.04	0.03 $\pm$ 0.01	0.04 $\pm$ 0.02

Table 6. *Effect of the administration of DL-propargylglycine for 3 days on the 24 h urinary excretion of sulphate and taurine from L-[35 S]cysteine by the rat*

Experimental details are given in the text. Values are means $\pm$ S.D. for four rats. Statistical significance was determined by Student's *t* test; * indicates that the +propargylglycine value is different than that for the control group fed *ad libitum* ($P < 0.05$), and † indicates that the +propargylglycine value is different than that for the pair-fed control group ($P < 0.05$).

Dietary treatment	Intubated labelled diet	Body-weight change (g/2 days)	Food intake (g/2 days)	Urinary excretion (% of absorbed dose)			[^{35}S]Taurine/ $^{35}\text{SO}_4^{2-}$ excretion ratio
				Total ^{35}S	$^{35}\text{SO}_4^{2-}$	[^{35}S]Taurine	
Control fed <i>ad libitum</i>	0.2% cysteine	14 $\pm$ 1	28 $\pm$ 4	6.3 $\pm$ 3.0	3.2 $\pm$ 1.6	0.84 $\pm$ 0.33	0.30 $\pm$ 0.17
Pair-fed control	0.2% cysteine	0 $\pm$ 4	16 $\pm$ 2	6.8 $\pm$ 3.0	3.9 $\pm$ 2.0	0.54 $\pm$ 0.50	0.12 $\pm$ 0.05
+Propargylglycine	0.2% cysteine + propargylglycine	-8 $\pm$ 1*†	10 $\pm$ 3*†	7.4 $\pm$ 0.8	3.4 $\pm$ 0.8	0.25 $\pm$ 0.05*	0.08 $\pm$ 0.03*
Control fed <i>ad libitum</i>	2.6% cysteine	17 $\pm$ 2	31 $\pm$ 4	47 $\pm$ 0.7	29 $\pm$ 1.6	9.3 $\pm$ 1.9	0.33 $\pm$ 0.08
Pair-fed control	2.6% cysteine	-5 $\pm$ 4	15 $\pm$ 2	60 $\pm$ 10	46 $\pm$ 12	5.6 $\pm$ 1.2	0.13 $\pm$ 0.03
+Propargylglycine	2.6% cysteine + propargylglycine	-7 $\pm$ 1*	13 $\pm$ 3*	50 $\pm$ 24	37 $\pm$ 18	7.1 $\pm$ 2.5	0.21 $\pm$ 0.09

Table 7. Effect of the administration of DL-propargylglycine for 3 days on cysteine desulphhydration and cystathionine γ -lyase activity in rat liver and kidney

Experimental details are given in the text. Values are means $\pm$ S.D. for three rats. Statistical significance was determined by Student's *t* test; * indicates that the +propargylglycine value is different than that for the control group fed *ad libitum* ($P < 0.05$), and † indicates that the +propargylglycine value is different than that for the pair-fed control group ($P < 0.05$).

Dietary treatment		Body-weight change (g/2 days)	Food intake (g/2 days)	Cysteine desulphhydration assay I (nmol of H ₂ S/min per mg of protein)		Cystathionine γ -lyase (nmol of 2-oxobutyrate/min per mg of protein)	
				Liver	Kidney	Liver	Kidney
Control fed <i>ad libitum</i>	Intubated diet 0.2% cysteine	15 $\pm$ 2	35 $\pm$ 12	2.2 $\pm$ 0.7	1.1 $\pm$ 0.1	5.0 $\pm$ 0.5	2.5 $\pm$ 0.2
Pair-fed control	0.2% cysteine	0 $\pm$ 2	16 $\pm$ 4	2.0 $\pm$ 0.1	1.1 $\pm$ 0.1	4.2 $\pm$ 0.6	2.6 $\pm$ 0.1
+Propargylglycine	0.2% cysteine + propargylglycine	-9 $\pm$ 1*	17 $\pm$ 5	0.13 $\pm$ 0.09*†	0.24 $\pm$ 0.07*†	0.22 $\pm$ 0.22*†	0.57 $\pm$ 0.20*†
Control fed <i>ad libitum</i>	2.6% cysteine	14 $\pm$ 1	38 $\pm$ 9	2.1 $\pm$ 0.6	1.0 $\pm$ 0.1	4.4 $\pm$ 1.1	2.1 $\pm$ 0.3
Pair-fed control	2.6% cysteine	0 $\pm$ 8	17 $\pm$ 1	2.2 $\pm$ 0.2	1.0 $\pm$ 0.1	4.9 $\pm$ 0.7	2.4 $\pm$ 0.4
+Propargylglycine	2.6% cysteine + propargylglycine	-9 $\pm$ 1*	17 $\pm$ 1*	0.21 $\pm$ 0.06*†	0.26 $\pm$ 0.08*†	0.45 $\pm$ 0.32*†	0.78 $\pm$ 0.29*†

control rats fed *ad libitum* and the pair-fed control rats. The excretion of [³⁵S]taurine and the ratio of [³⁵S]taurine to ³⁵SO₄²⁻ in the 24 h urine collections of rats treated with propargylglycine and intubated with a diet that contained 0.2% cysteine were significantly less than those for control rats fed *ad libitum*. This was an unexpected finding and is probably related to the difference in food intake of the two groups, as the excretion of [³⁵S]taurine by rats given propargylglycine and pair-fed control rats was similar.

Table 7 summarizes the effect of administration of DL-propargylglycine on cystathionine γ -lyase activity and cysteine desulphhydration in assay system I for liver and kidney of rats treated in the same manner as those used in the ³⁵S-tracer study. In agreement with the results reported in Tables 4 and 5, both cystathionine γ -lyase activity and H₂S production (assay system I) from cysteine were markedly lower in rats given propargylglycine than in control rats.

Discussion

Our results are consistent with the catalysis of H₂S production from cysteine by cystathionine γ -lyase, cystathionine β -synthase and cysteine aminotransferase plus 3-mercaptopyruvate sulphurtransferase. When H₂S production by rat liver and kidney homogenates was assayed in incubation mixtures that contained 280 mM-cysteine and 3 mM-pyridoxal 5'-phosphate at pH 7.8 (assay system I), activity appeared to be due to the action of cystathionine γ -lyase and cystathionine β -synthase. The

inhibitory effect of propargylglycine and the activating effect of *S*-adenosylmethionine on H₂S formation under these assay conditions suggested that each enzyme catalysed a major portion of the cysteine desulphhydration, but the exact proportion of the H₂S production due to the action of each enzyme cannot be calculated from our data.

When H₂S production by rat liver and kidney homogenates was determined in reaction mixtures that contained 160 mM-cysteine, 2 mM-pyridoxal 5'-phosphate, 3 mM-2-oxoglutarate and 3 mM-dithiothreitol at pH 9.7 (assay system II), activity was due almost entirely, if not entirely, to the combined activities of cysteine:2-oxoglutarate aminotransferase and 3-mercaptopyruvate sulphurtransferase. Neither propargylglycine nor *S*-adenosylmethionine had any effect on H₂S production in this system, whereas omission of 2-oxoglutarate from the reaction mixture for assay II decreased the activity by more than 90%. The tissue distribution, the concentrations of substrates and cofactors and the incubation pH required to yield a maximal rate of H₂S production and the actual values for the activity in various tissues closely parallel those found for cysteine aminotransferase activity (S.-M. Kuo, T. C. Lea & M. H. Stipanuk, unpublished work).

With both of the more-physiological assay systems, III and IV, addition or omission of 2-oxoglutarate had little effect on the H₂S production observed, whereas omission of pyridoxal 5'-phosphate from the reaction mixtures and treatment of tissue homogenates with propargylglycine and with *S*-adenosylmethionine had relatively large and signi-

ficant effects. Hence both cystathionine γ -lyase and cystathionine β -synthase appeared to be active in the catalysis of cysteine desulphhydration in these more-physiological systems, whereas cysteine aminotransferase, which has a much higher pH optimum (Ubuka *et al.*, 1978), did not appear to be involved.

A greater inhibition of H_2S production by propargylglycine in liver than in kidney was consistently observed. This suggested that a greater proportion of the H_2S production was catalysed by cystathionine γ -lyase in liver than in kidney, but the results of treatment of the tissue homogenates with *S*-adenosylmethionine did not suggest a greater relative role of cystathionine β -synthase in the catalysis of cysteine desulphhydration in kidney than in liver. A possible explanation for this observation is that *S*-adenosylmethionine was not specific for increasing cystathionine β -synthase-catalysed cysteine desulphhydration and increased cysteine desulphhydration by other pathways.

The physiological significance of cysteine desulphhydration is difficult to assess. Inhibition of cystathionine γ -lyase in the intact rat by propargylglycine had no significant effect on urinary excretion of ^{35}S , $^{35}SO_4^{2-}$ and $[^{35}S]$ taurine formed from labelled dietary cysteine. The desulphhydration of cysteine may not be a quantitatively large route of cysteine catabolism even though it may be physiologically important as a means of generating metabolically active reduced sulphur, or flux through other pathways leading to similar end products may increase when cystathionine γ -lyase activity is inhibited. There are several possible routes of sulphate formation from cysteine, which include both oxidation of sulphide formed by the desulphhydration pathways considered here and oxidation of sulphite released by desulphination of the 3-sulphinylpyruvate formed by transamination or oxidative deamination of cysteinesulphinate. The oxidation of cysteine to cysteinesulphinate followed by decarboxylation of cysteinesulphinate to hypotaurine or conversion of cysteinesulphinate into 3-sulphinylpyruvate is thought by many investigators to be the major pathway of cysteine degradation in animal tissues (Wheldrake & Pasternak, 1967; Yamaguchi *et al.*, 1973; Stipanuk, 1979).

The desulphhydration of cysteine appears to be catalysed by enzymes that are also physiologically active with other substrates. Cystathionine β -synthase apparently has a much higher K_m for L-cysteine (36 mM) than for L-serine (2–8 mM) or L-homocysteine (0.1–9 mM) (Nakagawa & Kimura, 1968; Braunstein *et al.*, 1969; Kraus *et al.*, 1978). The K_m of cysteine: 2-oxoglutarate aminotransferase for L-cysteine is about 22 mM, whereas its K_m for aspartate is about 0.5 mM (Ubuka *et al.*, 1978). In contrast, the K_m of rat liver cystathionine γ -lyase

for L-cystine has been estimated to be about 0.07 mM compared with 0.8–3.5 mM for L-cystathionine and 15–20 mM for L-homoserine (Uren *et al.*, 1978; Yao *et al.*, 1979). Thus cystine may be able to compete favourably with other substrates for cystathionine γ -lyase even though intracellular cystine concentrations are low (Greenberg, 1975).

Although the physiological significance of cysteine desulphhydration reactions in mammalian tissues is not clear, our data indicate that cysteine desulphhydration is catalysed by cystathionine γ -lyase and cystathionine β -synthase and possibly by cysteine aminotransferase plus 3-mercaptopyruvate sulphurtransferase in liver and kidney incubation systems that contain 2 mM-L-cysteine and 0.05 mM-pyridoxal 5'-phosphate at pH 7.4. These incubation conditions approach physiological values for substrate concentrations and pH, and our results therefore suggest that these enzymes are active in catalysis of cysteine desulphhydration in the intact animal.

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