Biochemical Pharmacology, Vol. 25, pp. 2179–2185. Pergamon Press, 1976. Printed in Great Britain.

# MECHANISM OF THE PROTECTIVE ACTION OF CYSTEINE AND PENICILLAMINE AGAINST ACETALDEHYDE-INDUCED MITOCHONDRIAL INJURY*

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(*Received* 19 *December* 1975; *accepted* 20 *February* 1976)

**Abstract**—The inhibition of several mitochondrial functions by acetaldehyde, a metabolite of ethanol oxidation, was previously shown to be almost completely relieved by cysteine. To study the mechanism of this protective effect, several derivatives of cysteine were also tested. Free sulfhydryl and amino groups appear to be required for maximal protection against the inhibition by acetaldehyde since no protective effect was found with *N*-acetylcysteine or *S*-methylcysteine. Cysteine interacts readily with acetaldehyde whereas *N*-acetylcysteine and *S*-methylcysteine do not. Penicillamine (β,β-dimethylcysteine) also relieved the inhibition by acetaldehyde, but prior incubation of penicillamine with acetaldehyde was required. Penicillamine was not as effective as cysteine, a finding which correlates with the weaker interaction of penicillamine with acetaldehyde. The greater stability of penicillamine compared to cysteine may be an advantage in studies of the effects of sulfhydryl amino acids on acute and chronic effects of ethanol and acetaldehyde.

Acetaldehyde, a metabolite produced during ethanol oxidation, inhibits several mitochondrial functions [1–7]. Cysteine, *in vitro*, affords almost complete relief of the acetaldehyde-induced inhibition of mitochondrial functions [8]. Cysteine probably exerts its protective effect by forming an adduct with acetaldehyde, thereby preventing acetaldehyde from interacting with the mitochondria [8]. To study the mechanism whereby cysteine protects against acetaldehyde-induced mitochondrial injury, the ability of several derivatives of cysteine, including *S*-methylcysteine, in which the sulfhydryl group is in thioether linkage with a methyl group, and *N*-acetylcysteine, in which one of the amino hydrogens is replaced by an acetyl group, to relieve the inhibitions by acetaldehyde was tested.

Cysteine, *in vivo*, is rapidly oxidized by amino acid oxidase or metabolized by cysteine desulfhydrase [9]. Thus, a more stable sulfhydryl-containing amino acid, which could interact with acetaldehyde, appeared to be a prerequisite for studies *in vivo*. We, therefore, also evaluated the effectiveness of penicillamine (β,β-dimethylcysteine) in relieving the acetaldehyde-induced inhibition of mitochondrial functions. This compound is more stable than cysteine [9], and has been used therapeutically as a copper-chelating agent in the treatment of Wilson's disease [10–12], and as an antidote in heavy metal intoxication [13]. Penicillamine may offer the advantages of stability, low toxicity and well-characterized biochemical and pharmacologic properties [14–17].

* These studies were supported in part by USPHS Grant AA287 and AA316.

† Recipient of Career Development Award (1 K02 AA 000003-01) from the National Institute on Alcohol Abuse and Alcoholism.

## METHODS

*Preparations.* Rat liver mitochondria were prepared as previously described [5]. Characterization of the acetaldehyde, preparation of the sulfur-containing agents, scintillation counting and protein determinations were as previously described [8]. The sulfur-containing agents (Sigma Chemical Co.) were all freshly prepared, neutralized, purged with nitrogen, stoppered and kept on ice.

*Oxygen consumption.* Oxygen uptake was assayed as previously described [5, 6, 8, 18] at 30° using a Clark oxygen electrode and a Yellow Springs oxygen monitor. The reaction system, equilibrated with air, consisted of 0.3 M mannitol; 10 mM Tris–HCl, pH 7.4; 10 mM potassium phosphate, pH 7.4; 2.5 mM $MgCl_2$; 10 mM KCl; and mitochondria equivalent to 3–4 mg protein in a final volume of 3.0 ml. Glutamate (10 mM) was the substrate in all experiments. ADP (1 mM) was added via a syringe to initiate state 3 conditions.

*$^{14}CO_2$ production.* $^{14}CO_2$ production was assayed using either 67 μM octanoate[1-$^{14}$C], 67 μM palmitate[1-$^{14}$C], 6.7 mM sodium succinate[1,4-$^{14}$C] or 6.7 mM sodium glutamate[1-$^{14}$C] (New England Nuclear) as the substrates. The reaction was carried out in flasks containing center well cups, using the reaction mixture described above for the oxygen consumption studies, along with 5 mM ADP, and mitochondria equivalent to about 2 mg protein, in a final volume of 3.0 ml. For the experiments with fatty acids, 3 mM ATP, 3 mM carnitine, 0.1 mM malate and 6 mg fatty acid-depleted bovine serum albumin [19] were added. The reaction was initiated by the addition of the labeled substrate, the flasks were stoppered, and the reaction was terminated after 15 min at 30° by the addition of 1 ml of 2 N HCl. Hydroxide of hyamine (0.3 ml) was injected into the center well, and $CO_2$ was allowed to collect in the center well

for 60 min. The cups were removed and placed in 10 ml Econofluor, and counted in a liquid scintillation counter.

*Interaction of acetaldehyde and thiols.* The sulfur-containing agent (400 nmoles) was incubated in 4 ml of 100 mM Tris–HCl, pH 8.5, with various amounts of acetaldehyde for 5, 10 or 20 min. 5-5'-Dithionitrobenzoic acid (final concentration of 0.25 mM) was then added, and the absorbance at 412 nm was recorded in a Gilford 240 spectrophotometer [20]. A mM extinction coefficient of 13.6 was used to calculate the concentrations of the thiols. Blanks included samples containing acetaldehyde but lacking the thiol or thioether.

Acetaldehyde (450 nmoles) was incubated in 4 ml of 100 mM potassium phosphate, pH 7.4, with various amounts of the sulfur-containing agent for 5, 10 or 20 min. Semicarbazide (15 mM in 160 mM phosphate buffer) was then added, and the absorbance at 224 nm was recorded [21]. A mM extinction coefficient of 9.4 was used to calculate the concentration of acetaldehyde. Blanks included samples containing the thiol but lacking acetaldehyde.

*Statistics.* All values represent the mean ± standard error of the mean. The number of experiments is indicated in the individual tables. Statistical analysis was performed by Student's *t*-test. In the case of samples containing acetaldehyde plus the sulfur-containing compound, the analysis was made against controls which lacked these two compounds, as well as samples which contained only acetaldehyde.

## RESULTS

*Effect of sulfur-containing agents on acetaldehyde-induced inhibition of glutamate oxidation.* None of the sulfur-containing agents tested in the absence of acetaldehyde had any effect on the state 3 rates of oxygen uptake associated with the oxidation of glutamate. A slight increase in the state 4 rate of oxygen uptake was observed in the presence of high concentrations of cysteine, but not with the other compounds. The state 4 rate of oxygen consumption was not affected by 3 mM acetaldehyde, whereas ADP-dependent oxygen uptake was depressed 40 per cent (Table 1). As has been shown previously [8], cysteine almost completely relieved this inhibition by acetaldehyde (Table 1). This protective effect was not observed with *N*-acetylcysteine or *S*-methylcysteine (Table 1). With D or DL-penicillamine a slight trend toward relief was noted, but the extent was much less than that found with cysteine.

In these experiments, the mitochondria were incubated with the sulfur-containing agent for 1–2 min in the polarograph chamber prior to the addition of acetaldehyde. The addition of cysteine after incubating mitochondria with acetaldehyde was much less effective than when cysteine was initially present in the incubation medium [8]. This suggested that the longer the interaction of cysteine with acetaldehyde, the greater the relief. Therefore, acetaldehyde was incubated either by itself or with 10 mM of the sulfur-containing reagents in an ice bath for 10 min, before

Table 1. Effect of sulfur-amino acids on acetaldehyde-induced inhibition of oxygen consumption*

| Respiratory state | Acetaldehyde | Addition | Concn (mM) | Oxygen uptake (natoms/min/mg protein) | Effect on control (%) | P | Effect on acetaldehyde (%) | P |
|---|---|---|---|---|---|---|---|---|
| 4 | – | | | 13.37 ± 1.19 | | | | |
| | + | | | 12.29 ± 1.25 | −8 | NS | | |
| | + | Cysteine | 10 | 16.27 ± 1.27 | +22 | < 0.01 | +32 | < 0.01 |
| | | | 20 | 17.28 ± 2.50 | +29 | < 0.02 | +41 | < 0.02 |
| | + | DL-Penicillamine | 10 | 12.23 ± 0.25 | −9 | NS | 0 | NS |
| | | | 20 | 13.06 ± 1.62 | −2 | NS | +6 | NS |
| | + | D-Penicillamine | 10 | 12.47 ± 0.65 | −7 | NS | +1 | NS |
| | | | 20 | 13.50 ± 1.80 | +1 | NS | +10 | NS |
| | + | *N*-acetylcysteine | 10 | 11.87 ± 0.91 | −11 | NS | −3 | NS |
| | | | 20 | 13.46 ± 1.44 | 0 | NS | +10 | NS |
| | + | *S*-methylcysteine | 10 | 11.63 ± 1.56 | −13 | NS | −5 | NS |
| | | | 20 | 12.90 ± 1.65 | −4 | NS | +3 | NS |
| 3 | – | | | 66.21 ± 4.39 | | | | |
| | + | | | 39.74 ± 4.15 | −40 | < 0.001 | | |
| | + | Cysteine | 10 | 54.28 ± 4.60 | −18 | 0.06 | +37 | 0.03 |
| | | | 20 | 59.67 ± 3.45 | −10 | NS | +50 | 0.02 |
| | + | DL-Penicillamine | 10 | 44.24 ± 4.64 | −33 | 0.003 | +11 | NS |
| | | | 20 | 45.70 ± 3.25 | −31 | < 0.01 | +15 | NS |
| | + | D-Penicillamine | 10 | 43.24 ± 4.22 | −35 | 0.002 | +9 | NS |
| | | | 20 | 45.17 ± 2.13 | −32 | < 0.01 | +14 | NS |
| | + | *N*-acetylcysteine | 10 | 39.90 ± 3.95 | −40 | < 0.001 | 0 | NS |
| | | | 20 | 40.47 ± 2.78 | −39 | < 0.01 | +2 | NS |
| | + | *S*-methylcysteine | 10 | 38.52 ± 3.44 | −42 | < 0.001 | −3 | NS |
| | | | 20 | 36.03 ± 1.59 | −46 | < 0.001 | −9 | NS |

* Oxygen consumption was assayed as described in Methods using 10 mM glutamate as substrate. Mitochondria were added to the incubation mixture and allowed to equilibrate for 3 min. Glutamate (10 mM), sulfur-amino acid (10 and 20 mM) and acetaldehyde (3 mM), were added in rapid succession, oxygen consumption was recorded for about 2 min (state 4), and ADP was added to initiate state 3 conditions. Results are the means of five individual experiments.

NS = not significant.

Table 2. Effect of sulfur-amino acids on acetaldehyde-induced inhibition of state 3 oxygen consumption after preincubation*

| Acetaldehyde | Addition | Oxygen uptake (natoms/min/mg protein | Effect on control (%) | P control | Effect on acetaldehyde (%) | P acetaldehyde |
|---|---|---|---|---|---|---|
| − | | 71.45 ± 2.05 | | | | |
| + | | 44.80 ± 4.62 | −37 | < 0.001 | | |
| + | Cysteine | 66.35 ± 3.82 | −7 | NS | +48 | < 0.001 |
| + | DL-Penicillamine | 58.70 ± 5.85 | −18 | 0.03 | +31 | 0.037 |
| + | D-Penicillamine | 58.05 | −19 | | +30 | |
| + | *N*-acetylcysteine | 50.60 ± 3.12 | −29 | < 0.001 | +13 | NS |
| + | *S*-methylcysteine | 48.83 | −32 | | +9 | |

* The reaction was carried out after incubating either buffer alone, buffer plus acetaldehyde, or buffer, acetaldehyde and thiol for 10 min in an ice bath. Aliquots of the reaction mixture were transferred to the polarograph chamber, and mitochondria, glutamate and ADP added in rapid succession. The final concentration of acetaldehyde was 3 mM and that of the thiols, 10 mM. The same concentrations were used in the preincubations. Results are the means of four individual experiments, except for two experiments with D-penicillamine and *S*-methylcysteine.

NS = not significant.

transferring to the polarograph chamber. Acetaldehyde, incubated 10 min in the absence of any addition, inhibited state 3 glutamate oxidation (Table 2). Cysteine almost completely relieved this inhibition, whereas *S*-methylcysteine and *N*-acetylcysteine were again without effect (Table 2). However, under these conditions penicillamine exerted a protective effect (Table 2). In all the experiments described below, the sulfur-containing agents were incubated with acetaldehyde and the reaction mixture. Controls and flasks with acetaldehyde alone were treated in the same manner.

*Effect of sulfur-containing agents on acetaldehyde-induced inhibition of $CO_2$ production.* Acetaldehyde depressed $CO_2$ production from several different substrates, including octanoate, glutamate and succinate (Table 3). Cysteine completely prevented the inhibitory action with all these substrates, whereas *N*-acetylcysteine and *S*-methylcysteine were without any effect (Table 3). Under these conditions (10-min preincubation with acetaldehyde), penicillamine also protected against the inibition of $CO_2$ production from these substrates (Table 3).

Previous experiments suggested that amounts of cysteine at least equimolar to those of acetaldehyde were required for protection; maximum protection was observed in the presence of excess cysteine [8]. The ability of varying concentrations of D-penicillamine to relieve the inhibition of octanoate and palmitate oxidation by acetaldehyde is shown in Table 4. Little relief of the inhibition of octanoate oxidation occurred at lower penicillamine concentrations (Table

Table 3. Effect of sulfur-amino acids on acetaldehyde-induced inhibition of $CO_2$ production*

| Substrate | Acetaldehyde | Addition | Activity (nmoles/10 min/mg protein) | Effect on control (%) | Effect on acetaldehyde (%) |
|---|---|---|---|---|---|
| Octanoate | − | | 17.30 | | |
| | + | | 9.03 | −48 | |
| | + | Cysteine | 16.09 | −7 | +78 |
| | + | DL-Penicillamine | 14.13 | −18 | +56 |
| | + | D-Penicillamine | 13.34 | −23 | +48 |
| | + | *N*-acetylcysteine | 9.95 | −42 | +10 |
| | + | *S*-methylcysteine | 9.39 | −46 | +4 |
| Glutamate | − | | 91.8 | | |
| | + | | 56.8 | −38 | |
| | + | Cysteine | 93.6 | +2 | +65 |
| | + | DL-Penicillamine | 81.3 | −11 | +43 |
| | + | D-Penicillamine | 76.4 | −17 | +35 |
| | + | *N*-acetylcysteine | 57.3 | −38 | +1 |
| | + | *S*-methylcysteine | 53.0 | −42 | −7 |
| Succinate | − | | 129.9 | | |
| | + | | 76.9 | −41 | |
| | + | Cysteine | 135.2 | +4 | +76 |
| | + | DL-Penicillamine | 104.6 | −19 | +36 |
| | + | D-Penicillamine | 96.7 | −26 | +26 |
| | + | *N*-acetylcysteine | 84.1 | −35 | +9 |
| | + | *S*-methylcysteine | 81.4 | −37 | +6 |

* Production of $^{14}CO_2$ from octanoate[1-$^{14}$C], glutamate[1-$^{14}$C] or succinate[1,4-$^{14}$C] was assayed as described in Methods in the presence or absence of 2 mM acetaldehyde, or 2 mM acetaldehyde plus 10 mM addition. Results are the means of two individual experiments. A 10-min preincubation period was employed in all experiments.

Table 4. Effect of D-penicillamine on acetaldehyde-induced inhibition of $CO_2$ production from octanoate and palmitate*

| Substrate | Acetaldehyde | D-Penicillamine concn (mM) | $CO_2$ production (nmoles/10 min/mg protein) | Effect (%) | P |
|---|---|---|---|---|---|
| Octanoate | – | | 15.79 ± 0.87 | | |
| | + | | 8.28 ± 2.33 | −48 | 0.02 |
| | + | 0.5 | 9.52 ± 1.60 | −40 | 0.02 |
| | + | 1.0 | 9.89 ± 2.06 | −37 | 0.03 |
| | + | 3.3 | 11.34 ± 2.80 | −28 | 0.10 > P > 0.05 |
| | + | 10.0 | 13.05 ± 2.63 | −17 | NS |
| Palmitate | – | | 13.08 | | |
| | + | | 6.24 | −52 | |
| | + | 0.5 | 7.08 | −46 | |
| | + | 1.0 | 7.72 | −41 | |
| | + | 3.3 | 8.46 | −33 | |
| | + | 10.0 | 10.42 | −20 | |

* Production of $^{14}CO_2$ from octanoate[1-$^{14}$C] (three experiments) or palmitate[1-$^{14}$C] (one experiment) was assayed as described in Methods in the presence of either 2 mM acetaldehyde (octanoate experiments) or 1.5 mM acetaldehyde (palmitate experiment) and the indicated concentration of D-penicillamine. A 10-min preincubation period was employed in all experiments.

NS = not significant.

4), but greater relief was observed at higher concentrations (Table 4). Similar results were obtained with palmitate[1-$^{14}$C] as the substrate.

*Interaction of acetaldehyde with thiols and thioethers.* Acetaldehyde readily interacts with semicarbazide to form a semicarbazone, which is detected by its absorption at 224 nm. Interaction of acetaldehyde with the compounds studied in this report would be expected to decrease the amount of acetaldehyde that can be detected as the semicarbazone complex. Acetaldehyde was incubated with different amounts of these agents for various times, semicarbazide was then added, and the absorbance at 224 nm was recorded. Increasing amounts of cysteine progressively decreased the detectable content of acetaldehyde, particularly after prolonged incubation (Table 5). The cysteine–acetaldehyde complex appeared stable, since there was no release of acetaldehyde from the complex, in the presence of semicarbazide even after prolonged periods of incubation. There was no interaction of *S*-methylcysteine with acetaldehyde, even at very high ratios of this thioether to acetaldehyde, or after as much as 20 min of incubation (Table 5). This method could not be accurately used with *N*-acetylcysteine, because high blank values resulted from the interaction of semicarbazide with the carbonyl moiety of the acetyl group. Interaction of DL- and D-penicillamine with acetaldehyde was revealed by this method, with maximal interaction occurring after longer periods of incubation and higher penicillamine concentrations (Table 5). Cysteine reacts with acetaldehyde to a greater extent than penicillamine, as revealed by the lower amount of free acetaldehyde detected after incubation with cysteine, compared to that detected after incubation with penicillamine.

Free sulfhydryl groups can be readily detected by use of the reagent, dithionitrobenzoic acid (Ellman's reagent) [20]. Interaction of the sulfhydryl groups of a thiol with acetaldehyde should decrease the number of free sulfhydryl groups that can be detected with Ellman's reagent. The sulfur-containing compounds were incubated with amounts of acetaldehyde for different times, Ellman's reagent was then added, and the absorbance at 412 nm was recorded. Increasing amounts of acetaldehyde progressively decreased the detectable content of cysteine (Table 6), particularly at longer incubation periods. There was no interaction of acetaldehyde with *N*-acetylcysteine or *S*-methylcysteine, even after 20 min of incubation (Table 6). Hence, the ineffectiveness of these two compounds seems to be related to the lack of interaction with acetaldehyde. Surprisingly, the amount of penicillamine detected by this method was not influenced by acetaldehyde, contrary to the results obtained with the semicarbazide method (compare Tables 5 and 6). The reaction product formed between penicillamine and acetaldehyde is probably not as strong as that formed between cysteine and acetaldehyde, so that addition of Ellman's reagent liberates penicillamine (which then interacts with Ellman's reagent), but not cysteine. To verify the poor ability of *N*-acetylcysteine to interact with acetaldehyde by another method, acetaldehyde in 100 mM phosphate buffer, pH 7.4, was incubated with different amounts of *N*-acetylcysteine at 30°. The contents of the flasks were transferred to screw cap tubes and the acetaldehyde concentrations determined by gas chromatography. Under these conditions, more than 95 per cent of the acetaldehyde could be recovered as free acetaldehyde, with the same retention time as standard acetaldehyde. Less than 5 per cent of the acetaldehyde could be recovered when cysteine replaced *N*-acetylcysteine.

## DISCUSSION

Cysteine reversed the inhibition by acetaldehyde of several mitochondrial functions, probably by forming an adduct with acetaldehyde and thereby preventing the latter from interacting with the mitochondria [8]. Thio- and dithioacetals are readily formed between aldehydes and mercaptans, with subsequent ring closure to the thiazoldine-4-carboxylic acid [22–26]. In the case of acetaldehyde and cysteine, 2-methylthiazolidine-4-carboxylic acid is formed. Free sulfhydryl and

Table 5. Reaction of acetaldehyde and sulfur-amino acids*

| Acetaldehyde (nmoles) | Sulfur-amino acids | Ratio: addition/ acetaldehyde | Amount of acetaldehyde detected (nmoles) after incubation (min) 5 | 10 | 20 |
|---|---|---|---|---|---|
| 450 | Cysteine | | 435 | 470 | 450 |
| | | 0.5 | 411 | 371 | 340 |
| | | 1.0 | 370 | 340 | 320 |
| | | 2.0 | 331 | 292 | 200 |
| | | 5.0 | 284 | 176 | 127 |
| 450 | DL-Penicillamine | | 459 | 448 | 483 |
| | | 0.5 | 477 | 452 | 431 |
| | | 1.0 | 466 | 385 | 388 |
| | | 2.0 | 423 | 361 | 288 |
| | | 5.0 | 359 | 268 | 235 |
| | | 6.67 | 282 | 229 | 188 |
| 900 | DL-Penicillamine | | 899 | 870 | 887 |
| | | 0.5 | 843 | 819 | 747 |
| | | 1.0 | 731 | 670 | 579 |
| | | 2.0 | 617 | 521 | 356 |
| | | 3.3 | 485 | 360 | 250 |
| | | 6.67 | 332 | 289 | 229 |
| 450 | D-Penicillamine | | 444 | 448 | 477 |
| | | 0.5 | 427 | 424 | 396 |
| | | 1.0 | 401 | 368 | 245 |
| | | 2.0 | 350 | 337 | 276 |
| | | 5.0 | 369 | 295 | 209 |
| | | 10.0 | 286 | 234 | 189 |
| 450 | *S*-methylcysteine | | 478 | 468 | 464 |
| | | 0.5 | 462 | 501 | 471 |
| | | 1.0 | 481 | 458 | 513 |
| | | 2.0 | 496 | 452 | 501 |
| | | 5.0 | 467 | 469 | 484 |
| | | 10.0 | 441 | 425 | |

* Acetaldehyde was incubated with various amounts of sulfur-containing amino acids at 30° for 5, 10 or 20 min as described in Materials and Methods. Semicarbazide was added and the absorbance at 224 nm was recorded.

free amino groups in close proximity appear to provide maximal protection against the inhibitions by acetaldehyde, as evidenced by the fact that *S*-methylcysteine, in which the sulfhydryl group is bound in a thioether linkage, and *N*-acetylcysteine, in which one of the amino hydrogens is replaced by an acetyl group, have no protective effect. The lack of protection by these two derivatives of cysteine probably reflects the poor ability of these compounds to interact with acetaldehyde, at least in a stable manner under these conditions. Whereas *N*-acetylcysteine may interact with acetaldehyde to form the dithioacetal, ring closure to the thiazolidine derivative would not occur. In any event, using Ellman's reagent or gas chromatography, conditions which favored a strong stable interaction between cysteine and acetaldehyde did not promote such interaction between *N*-acetylcysteine and acetaldehyde. This correlates with the ability of cysteine, but not *N*-acetylcysteine, to relieve the inhibitions by acetaldehyde.

The inability of *S*-methylcysteine and *N*-acetylcysteine to provide relief against the acetaldehyde-induced inhibitions is in agreement with previous data suggesting that free sulfhydryl and amino groups are both required to provide significant protection. The need for the sulfhydryl group is suggested by the fact that compounds with free amino and free carboxyl groups (glycine or alanine) provide no relief of the acetaldehyde-induced inhibition [8]. The importance of the amino group is emphasized by the observation that thiols with free hydroxyl groups (mercaptoethanol) are less effective than cysteine [8]. $\beta$-Mercaptoethylamine is as effective as cysteine in relieving the inhibition by acetaldehyde, suggesting no role for the carboxyl group of cysteine in its protective action [8].

Under conditions which promote interaction, e.g. preincubation, high penicillamine to acetaldehyde ratios and no competition with mitochondrial receptors, penicillamine provided significant relief of the inhibitions by acetaldehyde. Penicillamine was not as effective as cysteine in relieving the acetaldehyde-induced inhibitions, possibly because of the weaker interaction of penicillamine with acetaldehyde and the fact that the adduct between cysteine and acetaldehyde is more stable than that between penicillamine and acetaldehyde. The lesser reactivity of penicillamine is due to the replacement of the hydrogens by methyl groups on the $\beta$-carbon of cysteine, which apparently leads to steric hindrance. Indeed, the use of penicillamine in Wilson's disease is made possible by the fact that it is sterically hindered relative to cysteine, so that a more stable copper chelate is formed with penicillamine [27, 28].

Since penicillamine is more stable than cysteine, this compound may be more suitable for studies *in*

Table 6. Reaction of sulfur-amino acids with acetaldehyde*

| Sulfur-amino acids | Amount added (nmoles) | Ratio: acetaldehyde/ addition | Amount of thiol detected (nmoles) after incubation (min) | | |
|---|---|---|---|---|---|
| | | | 5 | 10 | 20 |
| Cysteine | 400 | | 396 | 377 | 369 |
| | | 0.5 | 358 | 254 | 244 |
| | | 1.0 | 311 | 230 | 140 |
| | | 2.0 | 246 | 182 | 70 |
| | | 3.0 | 151 | 64 | 15 |
| DL-Penicillamine | 400 | | 354 | 350 | 314 |
| | | 0.5 | 340 | 342 | 318 |
| | | 1.0 | 348 | 332 | 328 |
| | | 2.0 | 336 | 356 | 306 |
| | | 3.0 | 334 | 326 | 314 |
| | | 5.0 | 344 | 314 | 276 |
| | | 10.0 | 364 | 306 | 288 |
| DL-Penicillamine | 800 | | 828 | 840 | 726 |
| | | 0.5 | 836 | 830 | 752 |
| | | 1.0 | 800 | 806 | 692 |
| | | 2.0 | 814 | 814 | 714 |
| | | 5.0 | 768 | 764 | 640 |
| | | 10.0 | 714 | 682 | 578 |
| D-Penicillamine | 400 | | 344 | 330 | 324 |
| | | 0.5 | 338 | 334 | 330 |
| | | 1.0 | 340 | 332 | 338 |
| | | 2.0 | 350 | 326 | 332 |
| | | 3.0 | 338 | 324 | 316 |
| | | 5.0 | 324 | 316 | 288 |
| *N*-acetylcysteine | 400 | | 414 | 406 | 406 |
| | | 0.5 | 404 | 398 | 430 |
| | | 1.0 | 392 | 386 | 404 |
| | | 2.0 | 428 | 390 | 418 |
| | | 3.0 | 420 | 394 | 416 |
| | | 5.0 | 414 | 366 | 412 |
| *S*-methylcysteine | 400 | | 354 | 356 | 366 |
| | | 0.5 | 334 | 336 | 326 |
| | | 1.0 | 328 | 334 | 342 |
| | | 2.0 | 334 | 330 | 332 |
| | | 3.0 | 338 | 332 | 330 |
| | | 5.0 | 338 | 324 | 318 |

* Sulfur-containing amino acids were incubated with various amounts of acetaldehyde for 5, 10 or 20 min, as described in Materials and Methods. Dithionitrobenzoic acid (Ellman's reagent) was added, and the absorbance at 412 nm was recorded.

*vivo* than cysteine. The administration of D-penicillamine and ethanol to rats gave rise to 2,5,5-trimethylthiazolidine-4-carboxylic acid in the urine [29]. This compound is most likely formed from the condensation of D-penicillamine with acetaldehyde derived from ethanol, with subsequent ring closure to the thiazolidine derivative. It is known that the toxicity of L-penicillamine, which is due to interaction with pyridoxal phosphate to form the thiazolidine ring structure, requires the presence of free sulfhydryl and amino groups in penicillamine [30]. The corresponding 2-methylthiazolidine-4-carboxylic acid could not be detected in the urine of rats treated with ethanol and L-cysteine [30]. Since cysteine *in vivo* protected rats against $LD_{90}$ doses of acetaldehyde [31], reduced ethanol-induced sleeping time [29], and readily interacts with acetaldehyde (Tables 5 and 6) [8], the lack of detection of 2-methylthiazolidine-4-carboxylic acid may reflect degradation *in vivo* of the reaction production between cysteine and acetaldehyde as opposed to lack of formation of the product. This complex can be oxidized slowly by mitochondria *in vitro* [25, 26]. Since D-penicillamine can divert part of the acetaldehyde generated during ethanol metabolism to urinary excretory products (2,5,5-trimethylthiazolidine-4-carboxylic acid) [29], and penicillamine and cysteine can protect against acetaldehyde-induced inhibition of mitochondrial functions *in vitro*, the possible protective effects of penicillamine and cysteine in acute and chronic ethanol intoxication warrant investigation.

*Acknowledgements* We thank Mr. M. Imam and A. Quereshi for expert technical assistance. We thank Dr. G. Cohen, Neurology Department, Mount Sinai School of Medicine, for performing the gas chromatography experiments.

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