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STRUCTURE–ACTIVITY RELATIONSHIPS OF CYSTEINE ESTERS AND THEIR EFFECTS ON THIOL LEVELS IN RAT LUNG *IN VITRO*

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Abstract—Pretreatment with cysteine esters increases cysteine (CySH) levels in rat lung and protects against the lethal effects of inhaled perfluoroisobutene *in vivo*. There are marked differences in the duration of protection achieved with different cysteine esters. In this study we have compared the uptake and metabolism of CySH, *N*-acetyl cysteine (NAc), cysteine esters and cystine esters *in vitro* using rat lung and liver homogenates and lung slices. Liver homogenates metabolized CySH and cysteine esters faster than lung homogenates. The half life ($T_{1/2}$) of CySH in lung was 58.8 ± 17.3 min and in liver was 14.0 ± 1.6 min (mean ± SEM). $T_{1/2}$ of the esters in lung ranged between 6.5 and 12.1 min and in liver between 1.9 and 5.3 min. Cysteine tertiary butyl ester, which does not protect *in vivo*, was not hydrolysed to CySH by lung or liver homogenates. All esters increased and prolonged intracellular CySH concentrations in lung slices to a much greater extent than CySH itself. NAc did not raise intracellular CySH above that of the controls and no NAc appeared within the slice. After CySH incubation intracellular CySH was 0.9 ± 0.1 nmol/mg wet wt at 10 min whereas after incubation with the esters it ranged between 2.60 and 3.65 nmol/mg wet wt. Cysteine cyclohexyl ester prolonged the increase of CySH the longest and cysteine methyl ester the shortest. CySH levels with cysteine cyclohexyl ester were 2.74 ± 0.15 and 4.13 ± 0.37 nmol/mg wet wt at 10 and 60 min, respectively, whereas with cysteine methyl ester, CySH levels were 2.60 ± 0.5 and 1.25 ± 0.08 nmol/mg wet wt at similar times. Cystine esters increased intracellular concentrations of both cystine and CySH. CySH concentrations ranged between 2.92 and 3.19 nmol/mg wet wt and cystine between 1.39 and 1.47 nmol/mg wet wt at 60 min. The elevation and duration of CySH in lung slices is well correlated with the duration of protection against perfluoroisobutene achieved *in vivo*.

The protection of tissues against toxic insult is mediated intracellularly by nucleophiles such as glutathione (GSH§). They may function by direct or enzyme mediated reaction with toxic electrophiles or indirectly by reaction with radicals, such as lipid hydroperoxides, to form oxidized GSH [1]. When the incoming toxicant is sufficiently reactive, cellular defence mechanisms may be quickly overwhelmed and the treatment of the poisoned individual is predominantly symptomatic and supportive. Such is the case in smoke inhalation and the ingestion of large doses of paracetamol [2] or paraquat [3]. If it was possible to identify the subject at risk it ought to be possible to pretreat with compounds that would raise intracellular levels of nucleophiles to reduce the toxic insult to the cell.

It has been reported that GSH monoethyl ester markedly increases intracellular concentrations of GSH [4–7] and protects mice against paracetamol toxicity [5], human lymphoid cells and skin fibroblasts against radiation damage [7] and prevents cataracts in newborn rats and mice [8]. GSH monoethyl ester was shown to increase tissue GSH probably by hydrolysis by cellular esterases [5]. *In vivo* GSH isopropyl ester raised lung and liver GSH levels and protected against paracetamol- and allyl alcohol-induced liver damage [9] and also against cadmium-induced kidney damage [10].

We have reported that cysteine methyl ester (CME) and cysteine isopropyl ester (CIPE) selectively increase cysteine (CySH) concentrations in rat lung after i.p. administration and these and other CySH esters prevent deaths of rats exposed to supra-lethal doses of the gaseous pulmonary oedemogen, perfluoroisobutene (PFIB) [11]. CIPE also protects mice from paracetamol toxicity in the liver and produces rapid but transient elevations of CySH in the liver, lung, kidney and spleen of the mouse, with the greatest elevation in the lung [12].

CySH esters were chosen for investigation, as previous studies had shown that at pH 7.5 the thiol group ionized to a greater extent than either CySH or GSH (16%, 6% and 1%, respectively). This suggests that a greater proportion of nucleophile would be available for reaction with a toxic electrophile [13]. In addition, increasing the lipophilicity of CySH by esterification might increase the uptake of the ester into tissue as cysteine ethyl ester (CEE) had been shown to have a high affinity for rat lung *in vivo* [14].

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§ Abbreviations: PFIB, perfluoroisobutene; CySH, cysteine; NAc, *N*-acetyl cysteine; GSH, glutathione; CME, cysteine methyl ester; CEE, cysteine ethyl ester; CIPE, cysteine isopropyl ester; CIBE, cysteine isobutyl ester; CCPE cysteine cyclopentyl ester; CCHE cysteine cyclohexyl ester; CTBE, cysteine tertiary butyl ester; CDME, cystine dimethyl ester; CDCHE, cystine dicyclohexyl ester; mBBr, monobromobimane; CRR, Cleland's Reductacryl Reagent.

R	Name	Abbreviation
—H	Cysteine	CySH
—CH_3	Cysteine methyl ester	CME
—CH_2—CH_3	Cysteine ethyl ester	CEE
—$(CH_2)_3$—CH_3	Cysteine butyl ester	CBE
—$CH(CH_3)_2$	Cysteine isopropyl ester	CIPE
—CH_2—$CH(CH_3)_2$	Cysteine isobutyl ester	CIBE
—$C(CH_3)_3$	Cysteine tertiary-butyl ester	CTBE
—C_5H_9	Cysteine cyclopentyl ester	CCPE
—C_6H_{11}	Cysteine cyclohexyl ester	CCHE

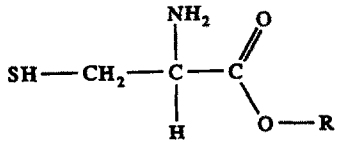

Fig. 1. Structures, names and abbreviations for CySH and CySH esters.

Recently we have used a rat lung slice model to evaluate the ability of potential CySH delivery systems, including two CySH esters, elevate CySH and replenish levels of GSH in the lung slice [15]. The CySH esters produced the greatest rise in CySH concentrations in the lung slice. In this study, we have investigated both the structure–activity relationships and mechanism(s) by which a series of CySH esters and cystine esters raise CySH levels in rat lung and liver homogenates and also their rates of uptake and metabolism in rat lung slices. The fate of the esters in slice medium has also been studied to establish whether the medium contributed significantly to the metabolism of the esters.

MATERIALS AND METHODS

Animals

Female Porton Wistar rats, 160–180 g, were used throughout these studies.

Chemicals

CySH and CME were purchased from the Sigma Chemical Co. (Poole, U.K.) and CRR (an immobilized form of Cleland's reagent, dithiothreitol, on a polyarylamide resin) from Calbiochem (Nottingham, U.K.). CEE, CIPE, cysteine isobutyl ester (CIBE), cysteine cyclopentyl ester (CCPE), cysteine cyclohexyl ester (CCHE), cysteine tertiary butyl ester (CTBE) (Fig. 1), cystine dimethyl ester (CDME), cystine dicyclohexyl ester (CDCHE) and monobromobimane (mBBr) were synthesized at the Chemical and Biological Defence Establishment. Pentobarbitone (Sagatal) was purchased from May and Baker Ltd (Dagenham, U.K.).

Homogenate studies

Preparation of lung and liver homogenates. Rats were humanely killed with Sagatal (300 mg/kg) and the lungs perfused with 0.9% saline [11] and the liver washed with 0.9% saline. The tissues were homogenized in Krebs physiological salt solution (Krebs) (118 mM NaCl, 1.0 mM $MgSO_4.7H_2O$, 1.2 mM KH_2PO_4, 4.8 mM KCl, 25 mM $NaHCO_3$, 2.6 mM $CaCl_2$ and 11.1 mM glucose) at pH 7.4 and centrifuged at 3000 rpm for 15 min. The supernatant fraction was removed and recentrifuged at 13,000 rpm (MSE Micro Centaur) for 5 min. The supernatant fraction contained mitochondria, microsomes, lysosomes and cytosol.

Preparation of plasma. Before perfusing the lungs, blood was withdrawn from the rat heart, heparinized and spun in Eppendorf tubes at 13,000 rpm for 5 min and the supernatant fraction removed.

Incubation of lung and liver homogenates and plasma with CySH and its esters. CySH or CySH esters and homogenized tissue (0.5 mL) were mixed using a vortex mixer and placed in a shaking water bath (Nickel Electro Ltd) at 37° and 150 strokes/min. The final concentration of added thiol was 1 mM and homogenized tissue 5 mg/mL (wet weight). Samples (50 μL) were taken immediately after mixing (no longer than 10 sec) at 1, 2, 5, 10, 15, 30 and 60 min. Undiluted plasma (0.5 mL) was incubated with 0.5 mL of thiol ester so that the final concentration of thiol was 1 mM.

Lung slice studies

Preparation of lung slices. The lungs were perfused as above and slices of 0.5 mm thickness were prepared on a McIlwain tissue slicer (the Mickle Laboratory Engineering Co. Ltd) and rinsed before use.

Incubation of the lung slices with CySH and its esters. Lung slices (27.2 ± 1.20 mg) were incubated in 5.0 mL of Krebs, pH 7.4, in the presence of 1 mM of selected thiol and gently agitated in a shaking water bath at 50 strokes/min and 37° [16–21]. At the required time points, 0, 10, 20, 30, 45 and 60 min, the lung slices and a 50 μL sample of the Krebs medium were removed and the thiols present derivatized with mBBr and assayed by HPLC as described below.

In a second series of experiments, the monosulphide esters (CME and CCHE) and the disulphide esters (CDME and CDCHE) were chosen in order to establish whether disulphides were being formed during incubation with lung slices. The esters were incubated with lung slices and the thiols present both in the slices and the media were assayed as described above. In addition after the required incubation time, 1.0 mL of media was removed and incubated with CRR. Lung slices were homogenized in 1.0 mL of Krebs and CRR. After 30 min incubation to ensure complete reduction of the thiols, the samples were centrifuged at 13,000 rpm for 5 min to remove the CRR. Samples (450 μL) of both medium and slice supernatant were derivatized as described below.

In an attempt to reduce the rate of ester disappearance and assess the contribution of soluble enzymes in the lung slice medium, lung slices were pre-incubated in Krebs for 5 min, gently washed and incubated in fresh Krebs for a further 5 min and gently washed again before incubation with 1 mM CIPE. Lung slices which had been thoroughly washed were incubated with CIPE for 60 min and

compared to those that had only been rinsed. To assess further whether soluble enzymes in the incubation medium were responsible for ester hydrolysis lung slices were incubated for 60 min in Krebs at 37° and 50 strokes/min and then the slices were either manually removed with forceps or the medium was filtered through No. 1 Whatman Filter Paper. CIPE (1 mM) was incubated with each of the filtered media in a volume of 5.0 mL.

Derivatization of thiols

Homogenates and medium. Homogenate or medium (50 μL) was removed and mixed rapidly with 850 μL of 0.1 mg/mL mBBr (made up in 0.05 M *N*-morpholine buffer) pH 8.0, incubated at 25° in the dark for 30 min until derivatized. To precipitate protein, 100 μL of 10% (v/v) methane sulphonic acid was added after derivatization and to remove any solid particulate material, the homogenate centrifuged at 13,000 rpm for 5 min (MSE Micro Centaur). A sample of the supernatant fraction was removed for HPLC analysis.

Slices. The slices were placed in 900 μL of 0.5 mg/mL mBBr, homogenized and incubated at 25° in the dark for 30 min until derivatized. Methane sulphonic acid (100 μL) was added and rapidly mixed. The homogenate was centrifuged at 13,000 rpm for 5 min (MSE Micro-Centaur).

Slices and CRR. The slices were place in 1.0 mL of Krebs and CRR, homogenized and incubated for 30 min. The incubate was then centrifuged at 13,000 rpm for 5 min to remove tissue and CRR. Supernatant fraction (450 μL) was added to 450 μL of 1.0 mg/mL and mBBr and derivatized as above.

Medium and CRR. One millilitre of medium was incubated with CRR for 30 min, centrifuged and 450 μL of the supernatant fraction derivatized in 450 μL of 1.0 mg/mL mBBr as above.

HPLC analysis. The method is one modified from Kosower *et al.* [22]. The equipment used: two Waters HPLC pumps, models 6000A and M510, Waters Automated Gradient Controller, Perkin Elmer LS5 Luminescence spectrometer, manual U6K injector or a Waters 712 WISP automatic injector and a Hewlett Packard 3393A Integrator. Column: HPLC Technology Hypersil 3ODS 150 mm × 4.6 mm with an Upchurch guard column packed with Perisorb RP18. CySH, GSH, *N*-acetyl cysteine (NAc), CME, CEE, CIPE and CIBE were separated by using a gradient controlled program which gave retention times of 2.80, 3.85, 7.05, 8.40, 8.80, 9.20 and 9.85 min, respectively. Two solvents were run simultaneously, solvent A, which was 0.25% HPLC glacial acetic acid in HPLC grade water (Rathburn Chemicals Ltd) adjusted to pH 3.7 and solvent B which was HPLC acetonitrile. The flow rate was 1.5 mL/min. A 10 μL sample was injected and run on a mobile phase of 92% A and 8% B for 4.5 min changed to 60% A and 40% B over 0.5 min. This continued for 6 min when the gradient returned to 92% A and 8% B over 1 min. Both changes of the solvent mixture in the mobile phase followed a linear gradient program. Five minute equilibration time was given before the next injection. To elute CCPE and CCHE the conditions were modified so that at 4.5 min the mobile phase changed to 35% A and 65% B. The retention times of CCPE and CCHE were 8.70 and 9.70 min, respectively.

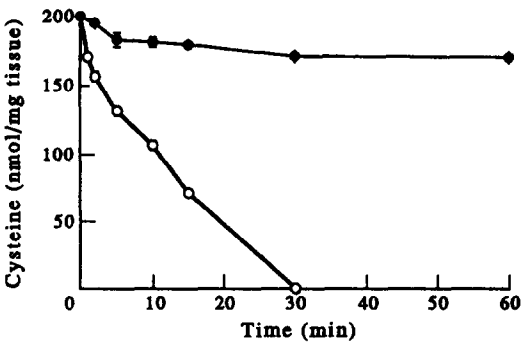

Fig. 2. Fate of CySH (1 mM) incubated in either lung (●) or liver (○) homogenates (5 mg wet wt/mL). Each point represents the mean (± SEM) of four animals. Duplicate aliquots were assayed at each time point.

Statistical analysis. The results were analysed by the Student's *t*-test for difference between two sample means where required.

RESULTS

Stability of CySH and its esters in the incubation medium

The stability of CySH and its esters (1 mM) in Krebs buffer, pH 7.4 and 37°, was measured. Less than 5% of all thiols was lost over the 60 min incubation period.

Homogenate studies

The fate of CySH and CySH esters when incubated for 60 min with lung and liver homogenates and plasma was compared.

Metabolism of CySH in lung and liver homogenates. The disappearance of CySH was much slower in lung homogenates (21% after 60 min) compared to liver homogenates (100% at 30 min) (Fig. 2).

Metabolism of the CySH esters in lung homogenates. When incubated in lung homogenates, all the CySH esters took in excess of 15 min to disappear completely with CySH levels rising slowly and remaining elevated (Fig. 3a–c) in most cases. CySH levels were highest with CME and CEE and lowest with CCPE and CCHE. The order of the rates of disappearance of the esters was CCHE > CIBE > CCPE > CEE > CIPE > CME. CTBE could not be determined as it did not form a fluorescent adduct with mBBr. However, it was not hydrolysed to CySH in lung homogenates.

Metabolism of the CySH esters in liver homogenates. The disappearance of the esters in liver homogenates was faster than in lung homogenates, with complete disappearance of all esters studied within 15 min. The CySH levels peaked between 10 and 15 min and fell rapidly. The order of the rate of disappearance of the esters was CIPE > CEE > CCHE > CIBE > CCPE > CME (Fig. 3d–f). CTBE was not hydrolysed to CySH by liver homogenates.

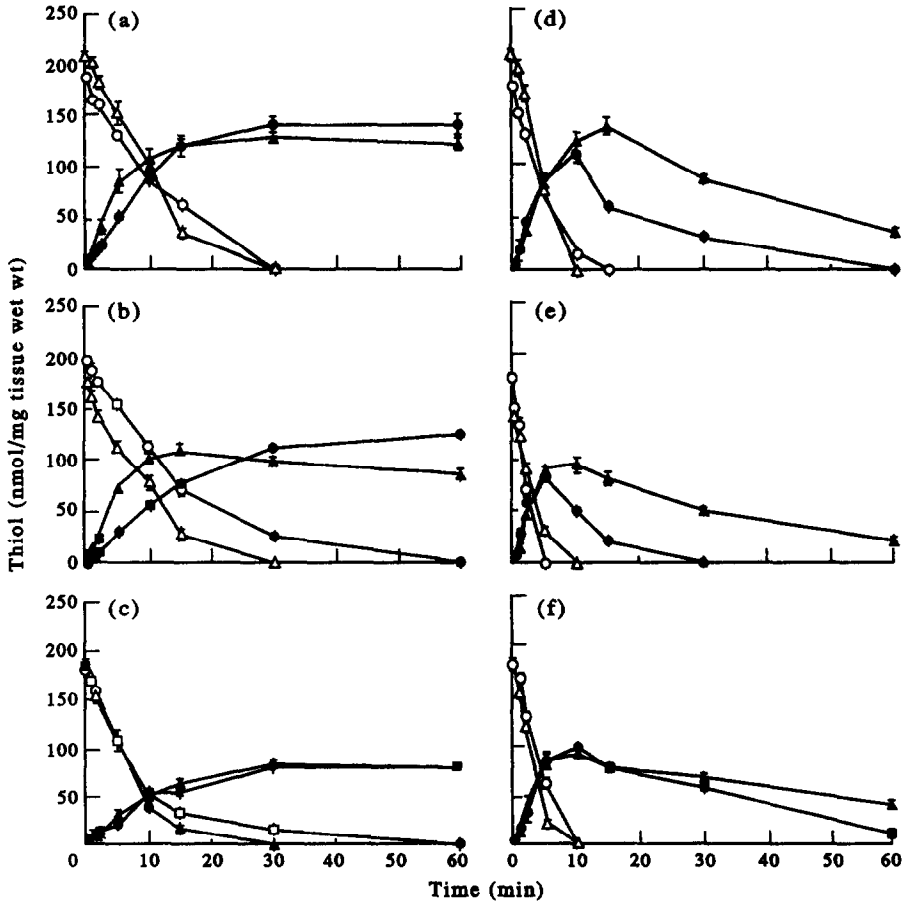

Fig. 3. Fate of cysteine esters (1 mM) incubated in lung homogenates (a–c) and liver homogenates (d–f) (5 mg wet wt/mL). Each point represents the mean (± SEM) of four animals. Duplicate aliquots were assayed at each time point. (a and d) CME (○); CySH (●); CEE (△); CySH (▲). (b and e) CIPE (○); CySH (●); CIBE (△); CySH (▲). (c and f) CCPE (○); CySH (●); CCHE (△); CySH (▲).

Metabolism of CySH and the CySH esters in plasma. CySH (1 mM) was rapidly metabolized in 50% plasma and had completely disappeared within 30 min. Esters incubated in plasma rapidly decreased and had completely disappeared within 15 min. CySH levels reached a maximum between 10 and 15 min and had fallen to zero by 60 min. CTBE was not hydrolysed to CySH by plasma. (Data not shown.)

Lung slice studies

The uptake and metabolism of CySH, NAc, CySH and cystine esters were determined in rat lung slices. Thiol levels were measured intracellularly and extracellularly by HPLC. The effect of rigorously washing the slices and oxidation of the CySH esters was studied.

Incubation of CySH and NAc with lung slices. CySH levels decreased by 18% after 60 min incubation with lung slices (Fig. 4a). At the same time CySH levels inside the slices rose 4-fold within 10 min (0.22 ± 0.03 to 0.88 ± 0.1 nmol/mg lung wet wt) and the levels remained constant between 45 min and 60 min (Fig. 4b). NAc levels fell by 8% after 60 min incubation with the lung slices (Fig. 4a). Extracellular CySH levels increased by a very small amount (20 nmol/mL) and intracellular levels increased by only 0.05 nmol/mg. GSH levels remained constant. After an initial fall due to leaching, GSH levels remain fairly constant at 0.7–0.75 nmol mg/lung wet wt. The control values were between 1.20 and 1.60 nmol/mg lung wet wt (Fig. 4d).

Incubation of CySH esters with lung slices. When CySH esters were incubated with lung slices the order of the rate of disappearance from the external medium was, CME > CBE > CIPE > CCPE > CIBE > CCHE (Fig. 4a). CySH levels inside the slice were elevated to similar levels after 10 min [CME at 2.60 ± 0.5 (lowest) and CIBE at 3.65 ± 0.14 (highest) nmol/mg lung wet wt, Fig. 4b]. However, at later time points CySH levels were higher and prolonged with CCHE and CCPE. CTBE, CIPE and CIBE all increased and prolonged CySH levels by a similar amount and CME produced the shortest duration of elevated CySH inside the slices. CySH levels were highest in the CME medium and lowest in the CCHE medium (Fig. 4c). After

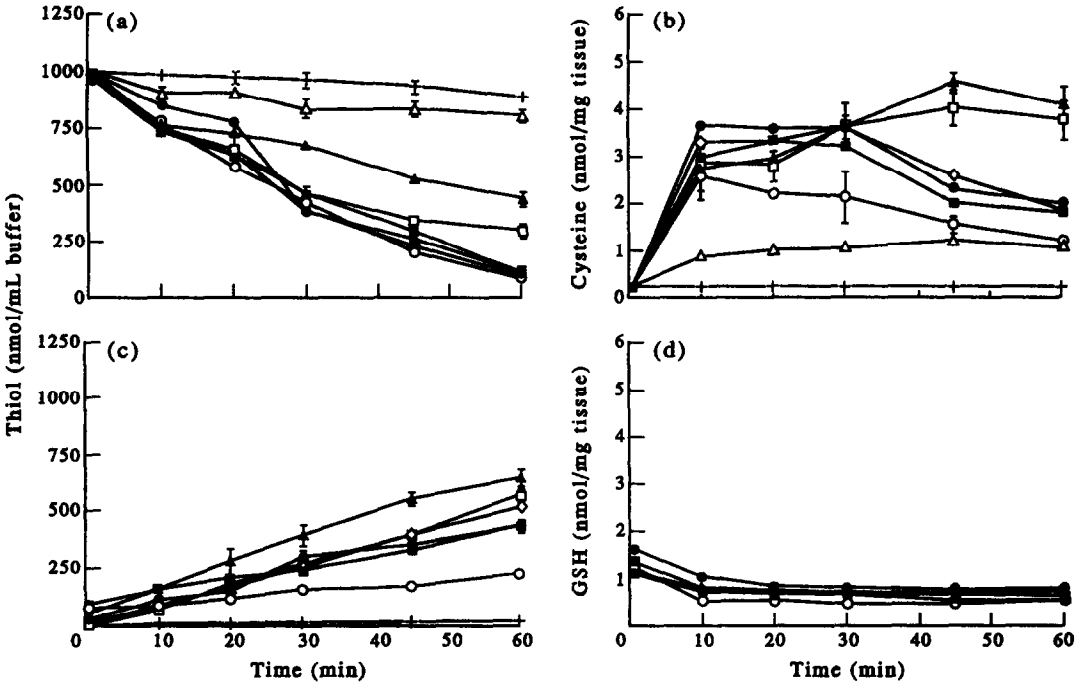

Fig. 4. (a) Disappearance of CySH and CySH esters (1 mM) from the slice medium containing rat lung slices (25–30 mg) incubated in Krebs physiological salt solution pH 7.4 and (c) the appearance of CySH in the same medium. (b) Elevation of CySH and (d) GSH levels inside the same lung slices. The results are expressed as the mean (± SEM) with three rats. CySH (△); NAc (+); CME (○); CBE (◊); CIPE (■); CIBE (●); CCPE (□); CCHE (▲).

an initial fall due to leaching GSH levels remained relatively constant at 0.7–0.75 nmol mg/lung wet wt. The control values were between 1.20 and 1.60 nmol/mg lung wet wt (Fig. 4d). CCHE could sometimes be detected intracellularly at concentrations up to a maximum of 0.1 nmol/mg lung wet wt or below but no other ester was detected inside the slice.

Effect of multiple washing of the lung slices. Lung slices, which had been thoroughly washed, were incubated with CIPE for 60 min and compared to those that had only been rinsed. The rate of disappearance of CIPE from the external medium was reduced when lung slices were rigorously washed compared to rinsed slices. ($P < 0.05$ at 45 and 60 min, respectively) (Fig. 5a). CySH concentrations inside the rigorously washed slices were maintained longer than the rinsed slices ($P < 0.05$ at 30 min, $P < 0.01$ at 45 and 60 min, respectively) (Fig. 5b). In the rinsed slices, GSH concentrations fell by a similar amount as the other studies (from 1.4 to 0.8 nmol/mg lung wet wt). However, with the rigorously washed slices GSH concentrations remained constant at 0.8 nmol/mg lung wet wt.

CIPE disappearance in filtered and unfiltered slice medium. Lung slices were incubated for 60 min and either removed manually or by filtration and CIPE was incubated with each of the filtered media. CIPE levels had decreased by 14.2 and 9.2% in the filtered and manually filtered media, respectively, after 60 min.

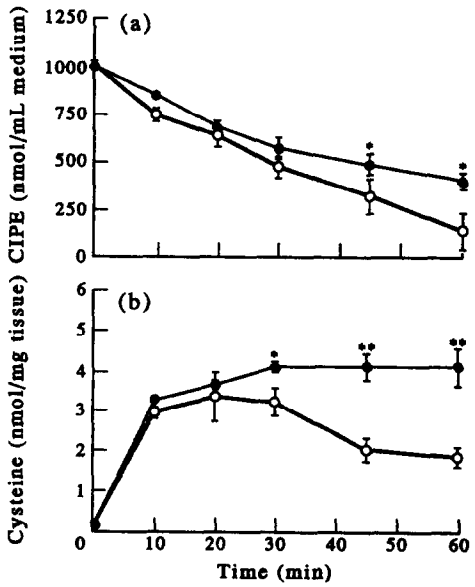

Fig. 5. Effect of multiple washing of lung slices upon (a) CIPE (1 mM) disappearance in the medium and (b) CySH levels inside the slice. The results are expressed as the mean (± SEM) with three rats. CIPE and CySH levels were compared at each time point; washed (●); rinsed (○); * $P < 0.05$; ** $P < 0.01$.

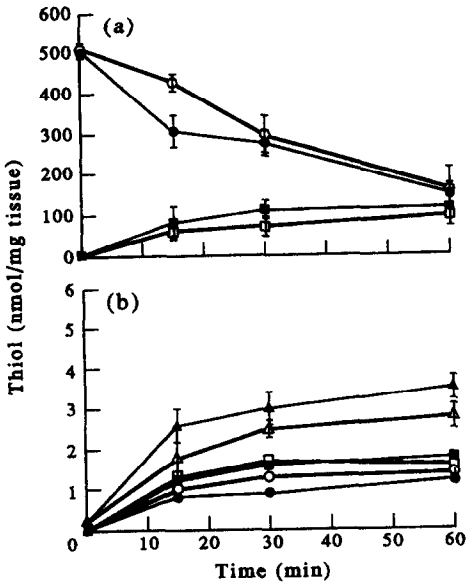

Fig. 6. (a) Disappearance of CDME and CDCHE (0.5 mM) from the slice medium containing rat lung slices (25–30 mg) incubated in Krebs physiological salt solution pH 7.4 and the appearance of cystine in the same medium. CDME (●); cystine (■); CDCHE (○); cystine (□). (b) Elevation of CySH, cystine and CDME and CDCHE inside the same lung slices. CDME (●); CySH (▲); cystine (■); CDCHE (○); CySH (△) cystine (□).

Incubation of CySH and cystine esters with CRR. To establish whether disulphides were present during the incubation of the monosulphide esters, CME and CCHE, with the lung slices, samples were assayed in the presence and absence of CRR. The disulphide esters CDME and CDCHE were also investigated.

No disulphides were detected either intracellularly in the slices or extracellularly in the medium following incubation with CME and CCHE (data not shown). In the extracellular medium CDME and CDCHE disappeared at a slower rate than their equivalent monoesters, with CDME disappearing faster than CDCHE. Cystine concentrations increased but no CySH or monoester was detected (Fig. 6a). CDME and CDCHE both raised intracellular levels of CySH (2.8 ± 0.3 and 3.5 ± 0.3 nmol/mg lung wet wt at 60 min, respectively). When assayed after treatment with CRR, CySH levels were increased by a further 2-fold from CySH produced by reduction of intracellular disulphides, probably the majority from the reduction of cystine. In contrast to the monoesters both CDME and CDCHE were found inside the slice (1.13–1.26 nmol/mg wet wt at 60 min, respectively) (Fig. 6b).

DISCUSSION

This study has shown that the lung has a limited capacity to metabolize CySH and this may explain why CySH concentrations can be raised to much higher levels in the lung than in other organs following *in vivo* pretreatment with various esters of CySH [11–13]. In addition the capacity of lung slices to take up CySH was more limited than that of the esters and intracellular CySH concentrations reached a steady state analogous to that reported for kidney and liver slices [23, 24]. Values for the control levels of CySH agree well with values reviewed by Bannai [25].

Lung slices have an even more limited capacity to take up NAc and this confirms previous studies *in vivo* which have shown that CySH levels in lung tissues are considerably lower after NAc administration than after CySH ester administration. Conversely plasma non-protein thiol levels were shown to be much higher after NAc administration than after ester administration [11]. This would indicate that although the lung slices have a limited capacity to metabolize NAc, its metabolism to CySH outside of the lung may be the main factor in determining the elevation of CySH within the lung.

CySH esters incubated with lung slices raised intracellular CySH levels more than following CySH incubation alone. Ester metabolism by lung slices followed a trend of the less lipophilic esters disappearing from the medium faster than the more lipophilic esters. When the esters had completely disappeared from the external medium, the intracellular concentration of CySH fell quickly to the same level as that produced by CySH. However with the slowly metabolized esters intracellular CySH levels remained elevated for the entire incubation period. The uptake of the CySH esters appeared to be an important factor controlling intracellular CySH levels, but when the ester levels decreased, the uptake of CySH becomes the main determinant of intracellular CySH levels. Esters are taken up either through the cell membrane where they are then hydrolysed to CySH or they are hydrolysed as they pass through the cell membrane. No intact esters were detected within the slice indicating that hydrolysis is very rapid after uptake.

The rate of disappearance of CIPE in the incubation medium was slower when the lung slices were thoroughly washed free of extraneous debris before incubation. Consequently the levels of CIPE remained higher for longer in the incubation medium and this was reflected in the prolongation of CySH levels intracellularly. Those esters which disappear slowest, e.g. CCHE, from the incubation medium are those which sustain the levels of intracellular CySH longest. This agrees well with the *in vivo* studies of Lailey *et al.* [11] in which lipophilic esters protected for up to 90 min against inhaled PFIB compared with less than 30 min for less lipophilic esters e.g. CME.

CySH is transported into and out of cells by two Na^+ dependent and one Na^+ independent carrier systems designated A, ASC and L, respectively, which operate in the direction of the concentration gradient. The ASC system is responsible for the transport of approximately 85% of the CySH in liver hepatocytes and kidney slices [25]. Studies from our laboratories indicate that the ASC system also functions in lung slices as it is both temperature and Na^+ concentration dependent. The total amount of

CySH within the slice at any time point represents a very small percentage of the total starting amount of ester since the export of CySH into the external medium appears to be highly efficient. It is proposed that most of the CySH present in the extracellular medium is derived from CySH exported from within the slice.

To determine whether disulphides contributed in the uptake and metabolism of the esters, CME and CCHE were incubated with lung slices and part of each sample was reduced with CRR before assay with mBBr. Disulphides were not detected intracellularly or extracellularly indicating that disulphides have little role to play in explaining the uptake and metabolism of the monosulphide CySH esters in the lung slice model. It does not exclude, however, that disulphides may be formed *in vivo*, particularly in the plasma.

The uptake of cystine is mediated by a different system to that of CySH. The dominant system is the Na^+-independent Xc^- system [25–30]. With the disappearance of the cystine esters from the external medium, intracellular concentrations of cystine and CySH increased and unlike the monoesters the cystine esters appeared intracellularly. In the external medium, cystine concentrations increased, probably exported by the Xc^- system. The presence of the cystine esters intracellularly would indicate that they diffused across the cell membrane where they were then hydrolysed to cystine which was then either exported to the external medium or reduced to CySH in the cell. Cystine esters are not converted to monosulphide esters in the lung slice preparation.

In this study lipophilic esters disappeared faster from homogenates but raised CySH concentrations least. As the metabolism of CySH is common to all preparations this implies that the more lipophilic esters are hydrolysed slower in agreement with the slice study. The other products of ester metabolism are not detected by the assay procedure. In lung slices non-hydrolytic pathways of ester metabolism are of minor importance as cell integrity is retained and uptake through the cell membrane and its associated ester hydrolysis predominate. Liver homogenates metabolized CySH and its esters faster than lung homogenates. This indicates that the activity of ester hydrolases in liver is much higher than in lung and that metabolism of CySH is faster. CTBE was not hydrolysed to CySH by the homogenates and also does not protect against supra-lethal doses of PFIB in the rat indicating that intracellular CySH concentrations need to be raised in order to protect.

In summary, CySH is metabolized faster by liver homogenates and plasma than by the lung homogenates and these tissues may be important determinants of the duration of ester effectiveness *in vivo*. There appears to be a good correlation between the duration of the protection of rats against the toxic effects of PFIB and the time for which levels of CySH are raised within rat lung slices. The production of CySH within the slice appears to be a result of the uptake of esters into the slice by a mechanism other than the normal transport system for CySH. It is probable that when inside, or during transfer through the cell membrane, the CySH esters are rapidly hydrolysed to CySH. As the concentration of CySH rises, it is rapidly exported from the cell, probably by the ASC CySH transport system. The ester with the longest duration of protection *in vivo* (CCHE) is hydrolysed slowest and maintains steady state intracellular CySH levels the longest. This suggests that the relative lipophilic characters of the esters are important components of the uptake process.

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