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A NOVEL ROLE FOR CARBOXYLESTERASE IN THE ELEVATION OF CELLULAR CYSTEINE BY ESTERS OF CYSTEINE

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Abstract—Esters of cysteine, such as cysteine *iso*propylester (CIPE) or cysteine cyclohexylester (CCHE), are efficient delivery systems for cysteine to cells. After enzymic cleavage, the esters of cysteine provide a source of cellular cysteine, which may support reduced glutathione (GSH) synthesis and/or act as a direct chemoprotectant. Reducing esterase activity of rat lung slices or isolated hepatocytes with paraoxon or bis(4-nitrophenyl) phosphate or by reducing the temperature to 4° dramatically altered the metabolism of esters of cysteine; the initial increase in cellular cysteine was slowed, the residency time of cysteine esters in the extracellular pool was prolonged without substantially enhancing the levels of intracellular ester. Incubation of lung slices with CIPE at 4° led to a marked increase in cellular cysteine, which prior inhibition of esterase activity abolished. Inhibiting the neutral amino acid uptake systems, ASC and L, while effecting the uptake of cysteine, did not reduce the elevation of cellular cysteine by CIPE. We propose that the elevation of cellular cysteine by esters of cysteine may be mediated by membrane associated esterase activity.

Cysteine is an amino acid necessary for the synthesis of proteins and the tripeptide glutathione (reduced glutathione GSH,‖ γ-glutamyl-cysteinylglycine) [1]. GSH has several metabolic and protective functions within cells [2]. The cellular concentrations of GSH (0.5–10 mM) exceed the level of cysteine (10–100 μM) [3] and hepatic GSH is proposed as a means of storing cysteine [1].

The rate of GSH biosynthesis is limited by the activity of γ-glutamylcysteine synthetase and the supply of cysteine. When intracellular GSH levels are normal, GSH exerts a negative feedback on γ-glutamylcysteine synthetase activity so preventing further synthesis [4]. When GSH levels are depleted, removing the inhibitory feedback, the supply of cysteine becomes a limiting factor, determining the rate of GSH biosynthesis. The K_m of γ-glutamylcysteine synthetase for cysteine is 0.3 mM [5].

Cysteine can be transported into cells by the A, ASC and L systems present for the uptake of neutral amino acids [6, 7]. The A and ASC systems are Na^+-dependent and the A system is sensitive to 2-(methylamino)isobutyric acid (MeIAB). The component of the Na^+-dependent uptake insensitive to MeIAB is classified as the ASC system [6, 7]. The L-system is characterized by being blocked by 2-amino(2,2,1)-heptane-2-carboxylic acid (BCH) in Na^+-free medium [6, 7].

Several methods have been utilized to elevate cellular cysteine in order to protect against the toxicity of various chemicals. In isolated hepatocytes, L-methionine can be used to generate cysteine via the cystathionine pathway, stimulating GSH synthesis and protecting against bromobenzene toxicity [8]. However the cystathionine pathway is largely restricted to the liver, so making extrahepatic organs dependent on circulating GSH released by the liver for their supply of cysteine [9]. *N*-Acetylcysteine is a widely used cysteine delivery system and is clinically proven as an effective treatment for acute paracetamol intoxication [10]. After deacetylation, it generates cysteine and supports GSH synthesis [11]. Alternatively, L-2-oxothiazolidine-4-carboxylic acid (OTZ) is converted to cysteine by 5-oxoprolinase [12] and also protects against the toxicity of paracetamol by stimulating the biosynthesis of GSH [13]. Recently, we have evaluated a number of novel esters of cysteine to act as chemoprotectants. It was reasoned that the increased lipophilicity of the esters relative to cysteine, would facilitate their entry into cells, thereby increasing the bioavailability of cysteine. Several esters of cysteine were effective in protecting against the pulmonary toxicity of inhaled perfluoroisobutene [14] and cysteine isopropylester protected against paracetamol-induced hepatotoxicity [15]. The proposed mechanism of protection was a direct interaction between reactive electrophiles and the esters and/or cysteine derived from hydrolysis of the esters. However, little or no intracellular ester was detected in either *in vivo* [14, 15] or *in vitro* [16] experiments, suggesting rapid hydrolysis by esterases. In this study, we have utilized a rat lung slice model

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‖ Abbreviations: GSH, reduced glutathione; CIPE, cysteine *iso*propylester; CCHE, cysteine cyclohexylester, MeIAB, 2-(methylamino) isobutyric acid; BCH, 2-amino(2,2,1)-heptane-2-carboxylic acid; OTZ, L-2-oxothiazolidine-4-carboxylic acid.

and isolated hepatocytes to investigate the role of esterases in the metabolism of cysteine *iso*propylester (CIPE) and cysteine cyclohexylester (CCHE) using the organophosphate inhibitors of esterase activity paraoxon and bis(4-nitrophenyl) phosphate. Inhibition of carboxylesterase activity dramatically slowed the hydrolysis of the cysteine esters by isolated hepatocytes and lung slices, without promoting intracellular ester concentrations and inhibition reduced the ability of the esters of cysteine to elevate intracellular cysteine. We propose a possible role for a membrane-bound esterase in the promotion of cellular cysteine by the esters of cysteine.

MATERIALS AND METHODS

Compounds. L-Cysteine isopropylester and L-cysteine cyclohexylester were synthesized as described previously [14]. Paraoxon was a generous gift of Dr M. K. Johnson (MRC Toxicology Unit, Surrey, U.K.). Bis(4-nitrophenyl) phosphate hydrate and 2(methylamino) isobutyric acid were obtained from the Aldrich Chemical Co. (Gillingham, U.K.). 2-Amino(2,2,1)-heptane-2-carboxylic acid was obtained from the Sigma Chemical Co. (Poole, U.K.). Monobromobimane (Thiolute®) was obtained from Calbiochem-Behring (Frankfurt, Germany).

Animals. Male Wistar rats (Porton strain) (180–250 g) were bred at the MRC Toxicology Unit animal facility. Animals were housed on a 12-hr light/dark cycle and allowed free access to food and water.

Preparation of rat lung slices. Lung slices were prepared as described by Hardwick *et al.* [17] and hepatocytes by the method of Seglen [18]. Lung slices or isolated hepatocytes were incubated with bis(4-nitrophenyl) phosphate (200 μM) or paraoxon (10 μM) for 30 min to inhibit esterase activity. Control slices and hepatocytes were treated in a similar manner but inhibitors of esterase activity were omitted. Control and esterase compromised slices were then incubated for up to 2 hr with CIPE or CCHE.

As paraoxon is susceptible to hydrolysis and inactivation by serum paraoxonase, lung slices and hepatocytes were prepared with and incubated in serum-free medium. Lung slices were incubated with MeIAB (25 mM) or in a Na^+-free medium or in Na^+-free medium with BCH (10 mM) with either CIPE or cysteine (500 μM) for 15 min to inhibit the A, ASC and L neutral amino acid transport systems. The osmolarity of the Na^+-free medium was maintained by replacing NaCl with choline chloride and Na_2PHO_4 buffer with K_2PHO_4 buffer.

Measurement of lung slice sulphydryls. The measurement of tissue sulphydryl content (cysteine, GSH, CIPE and CCHE) was made by HPLC analysis based upon the method outlined by Laily *et al.* [14]. Membrane permeable monobromobimane was used to derivatize pulmonary and hepatic thiols and form fluorescent adducts. Lung slices, hepatocytes (1×10^6 cells) or an aliquot of extracellular medium were mixed with monobromobimane (0.5 mg/mL). After 15 min the derivatization reaction was stopped by altering the pH to 1 with methane sulphonic acid (10% v/v) and HPLC performed as described previously [16].

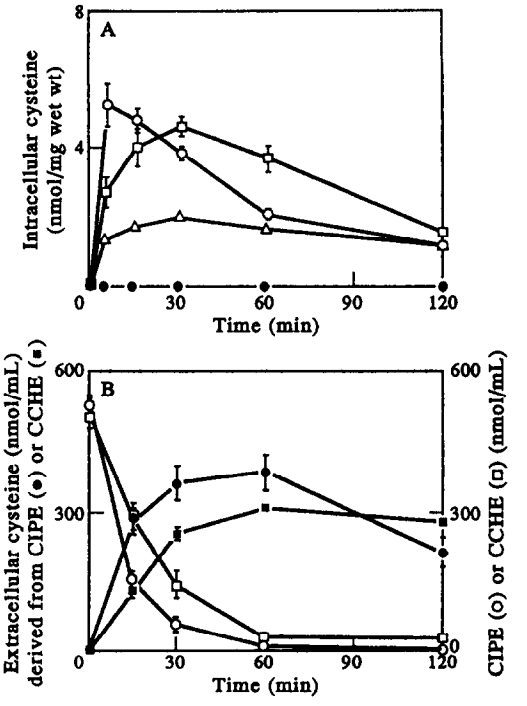

Fig. 1. The metabolism of CIPE, CCHE and cysteine by rat lung slices (A). Slices were incubated with CIPE (○), CCHE (□), cysteine (△) (500 μM) or received no treatment (●) for 120 min, and intracellular concentrations of cysteine assessed by HPLC. Extracellular levels of CIPE (○), CCHE (□) and cysteine derived from CIPE (●) or CCHE (■) (B) were also assessed by HPLC at the times indicated. Each point is the mean (± SEM) ($N \geq 3$).

RESULTS

Metabolism of CIPE and CCHE by rat lung slices after esterase inhibition

Addition of exogenous cysteine, CIPE or CCHE (500 μM) to rat lung slices caused a marked elevation in intracellular cysteine (Fig. 1). The elevation produced by both esters of cysteine was significantly greater than cysteine. The elevation in intracellular cysteine following incubation with both CIPE and CCHE was accompanied by a rapid disappearance of extracellular esters (<10% remained after 60 min) and the concomitant appearance of extracellular cysteine (Fig. 1B). Little (<1% of the ester added) or no unmetabolized CIPE or CCHE was detected intracellularly at any time. It was clear from these results that esterase activity was a major factor affecting the disposition of the esters. In order to investigate this, we used two inhibitors of esterase activity bis(4-nitrophenyl) phosphate and paraoxon.

Lung slices were preincubated with both inhibitors for 30 min as described, to inhibit esterase activity. This resulted in a marked alteration in the disposition

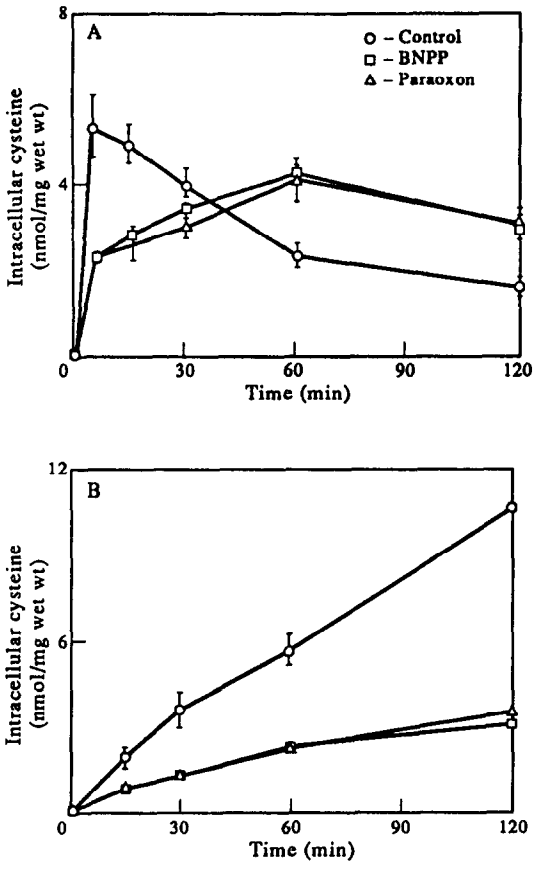

Fig. 2. Elevation of cellular cysteine by CIPE in control and lung slices with reduced esterase activity at 37° and 4°. Slices received no pretreatment (○) or were preincubated with paraoxon (10 μM) (△) or bis(4-nitrophenyl) phosphate (200 μM) (□) at 37° for 30 min. The slices were then transferred to fresh medium and incubated either at 37° (A) or 4° (B) with CIPE (500 μM). Each point is the mean of four to six separate experiments (± SEM).

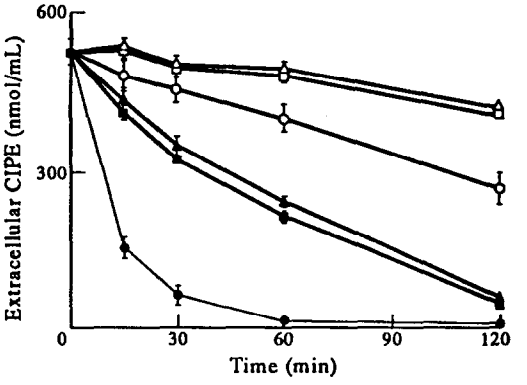

Fig. 3. Effects of temperature on extracellular CIPE in control and esterase-compromised lung slices. Slices received no pretreatment (○ or ●) or were preincubated with either paraoxon (10 μM) (△ or ▲) or bis(4-nitrophenyl) phosphate (200 μM) (□ or ■) before incubation with CIPE (500 μM) at either 37° (closed symbols) or 4° (open symbols). Extracellular CIPE was assessed by HPLC analysis. Each point is the mean of four to six experiments (± SEM).

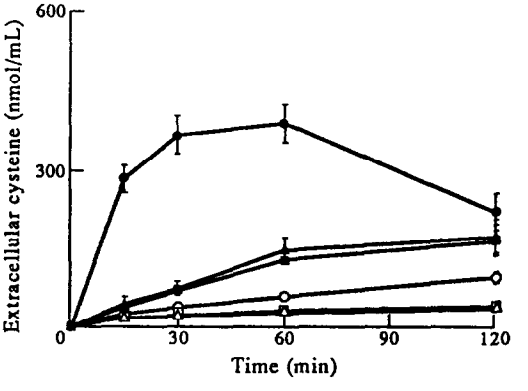

Fig. 4. Influence of esterase inhibition on the appearance of cysteine in the extracellular medium of lung slices incubated with CIPE at 4° or 37°. Slices received no pretreatment (○ or ●) or were preincubated with either paraoxon (10 μM) (△ or ▲) or bis(4-nitrophenyl) phosphate (200 μM) (□ or ■) before being exposed to CIPE at 4° (open symbols) or 37° (closed symbols). External concentrations of cysteine were measured by HPLC. Each point represents the mean of four to six experiments (± SEM).

of CIPE (Figs 2–4). The elevation of intracellular cysteine was slowed when esterase activity was inhibited (Fig. 2) but cellular cysteine levels continued to rise for a longer period (Fig. 2). The rates of hydrolysis of CIPE and CCHE to cysteine were inhibited, resulting in a prolonged half life of extracellular CIPE (Fig. 3 and Table 1) and a slower rise in extracellular cysteine (Fig. 4). After 60 min, 195 ± 15 nmol/mL CIPE (500 μM) remained compared to only 29 ± 5.5 nmol/mL when bis(4-nitrophenyl) phosphate was omitted. Similar results were obtained when control and esterase-compromised lung slices were incubated with CCHE (Table 1).

Despite the large pools of extracellular CIPE (Fig. 3) and CCHE due to esterase inhibition, only a slight elevation in the level of intracellular ester was detected (≤1% of the thiol added), which was considerably less than the elevation of intracellular cysteine observed when slices with intact esterase activity were incubated with CIPE or CCHE. This finding questions the role esterases play in the metabolism of the cysteine esters.

Elevation of intracellular cysteine by CIPE and CCHE at 4° after esterase inhibition

The rapid rate of intracellular cysteine enhancement by CIPE in control lung slices at 37° was slowed at 4° (Fig. 2). However, cysteine steadily accumulated over a 2-hr incubation period (Fig. 2). The continued rise in intracellular levels of cysteine was probably due to the continued presence of ester in the medium

Table 1. Inhibition of CCHE and CIPE hydrolysis by bis(4-nitrophenyl) phosphate, paraoxon and incubating at 4°

Pretreatment	CIPE 4°	CIPE 37°	CCHE 4°	CCHE 37°
Intracellular cysteine (nmol/mg wet wt)				
None	10.1 ± 1.3	1.34 ± 0.23	6.1 ± 0.96	1.57 ± 0.25
BNPP	2.8 ± 0.29	2.71 ± 0.28	3.7 ± 0.44	2.7 ± 0.13
Paraoxon	3.3 ± 0.05	2.80 ± 0.36	3.6 ± 0.09	2.5 ± 0.09
Extracellular cysteine (% thiol added)				
None	18.5 ± 2.0	43.3 ± 0.7	19.4 ± 1.5	59.6 ± 1.5
BNPP	7.5 ± 0.3	32.7 ± 5.6	16.9 ± 0.6	39.4 ± 1.4
Paraoxon	6.4 ± 0.5	33.7 ± 6.1	17.5 ± 1.2	42.8 ± 0.75
Extracellular ester (% thiol added)				
None	51.4 ± 5.0	0.3 ± 0.2	65.9 ± 2.4	5.9 ± 1.6
BNPP	79.5 ± 2.2	8.4 ± 1.8	75.0 ± 2.0	9.9 ± 1.2
Paraoxon	82.1 ± 2.6	10.2 ± 3.0	72.0 ± 7.3	8.8 ± 1.1

Rat lung slices received no pretreatment or were incubated with bis(4-nitrophenyl) phosphate (200 μM) or paraoxon (10 μM) for 30 min before exposure to CCHE or CIPE (500 μM) at 4° or 37°. After 2 hr, intracellular levels of cysteine and changes in the extracellular pools of cysteine and cysteine esters were assessed by HPLC.

Extracellular levels of cystine, CIPE and CCHE are expressed as a percentage of the ester added to the medium. (CIPE, 520 ± 23 and CCHE, 504 ± 35 nmol/mL). Each point represents the mean ± SEM (N = 3–9).

(Fig. 3). At 4°, slices with compromised esterase activity showed significantly lower levels of intracellular cysteine (Fig. 2B), despite high levels of extracellular CIPE or CCHE (Fig. 4). Again no dramatic rises in intracellular CIPE or CCHE were detected at 4° (not shown), levels were ≤1% of the sulphydryl added. The hydrolysis of CIPE and CCHE (500 μM) to cysteine in both control and esterase-compromised lung slices was markedly inhibited when incubated at 4° (Fig. 3). At 4° extracellular cysteine levels increased slowly (Fig. 4), mirroring the small drop in the level of extracellular ester. The hydrolysis of CIPE at 4° could be slowed even further by inhibiting esterase activity (Figs 3 and 4).

Slices incubated with cysteine (500 μM) at 4° had a reduced capacity to accumulate cysteine from the external medium (Fig. 5) (~50% reduction).

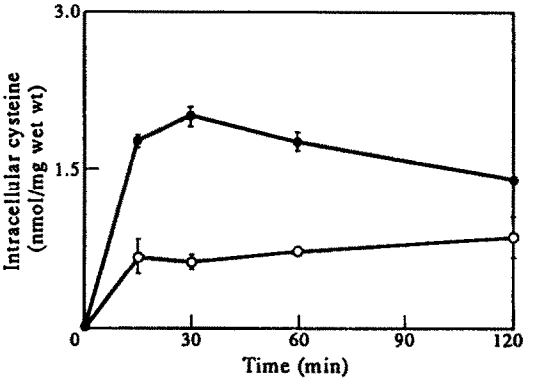

Fig. 5. The effect of temperature on the uptake of cysteine by rat lung slices. Slices were incubated with cysteine at 37° (●) or 4° (○) for up to 2 hr. Intracellular cysteine was measured by HPLC analysis. Each point represents the mean ± SEM of three experiments.

Metabolism of CIPE by isolated hepatocytes after esterase inhibition

The metabolism of CIPE (500 μM) by control rat hepatocytes was extremely rapid. Within 2 min, all the ester had been metabolized (Fig. 6C) and appeared primarily as extracellular cysteine (Fig. 6B). The metabolism of CIPE was coincident with a sudden, transient elevation in intracellular cysteine (Fig. 6A), far in excess of the levels achieved when incubated with cysteine alone. Inhibition of esterase activity with bis(4-nitrophenyl) phosphate or paraoxon prolonged the half life of CIPE in the extracellular medium (Fig. 6C) which was accompanied by a slow rise in extracellular cysteine (Fig. 6B) and a prolonged elevation in intracellular cysteine (Fig. 6A). No CIPE was detected intracellularly in control or esterase-compromised hepatocytes.

After 2 min, control hepatocytes incubated with CIPE were bathed solely in cysteine, which produced a slight elevation of intracellular levels of the amino acid (Fig. 6A). Cellular levels of GSH remained relatively constant over the 60-min incubation. All hepatocytes had similar viabilities as assessed by Trypan blue exclusion after incubations of 1 hr.

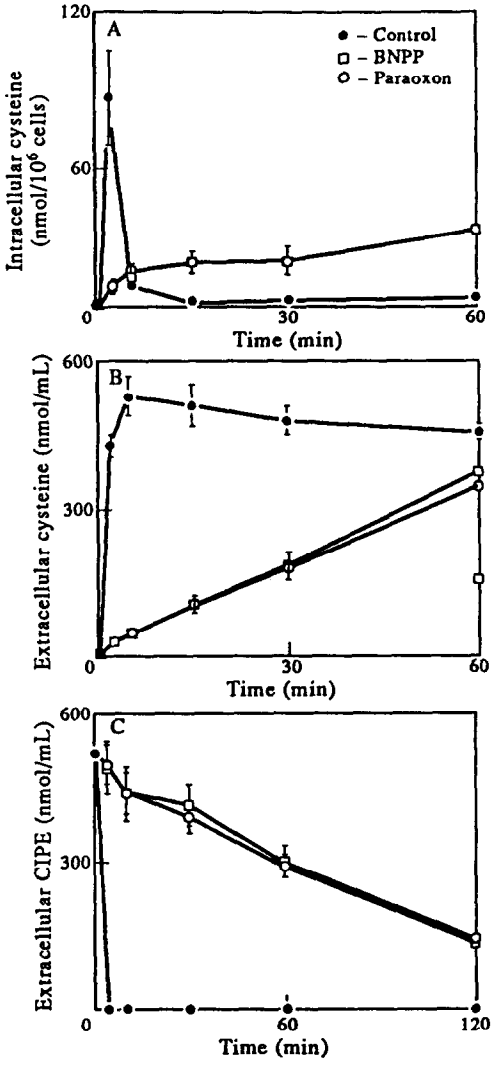

Fig. 6. Metabolism of CIPE by isolated rat hepatocytes with reduced esterase activity. Isolated hepatocytes (1×10^6 cells/mL) were preincubated for 30 min with paraoxon (10 μM) (○), bis(4-nitrophenyl) phosphate (200 μM) (□) or received no esterase inhibitor (●) before being treated with CIPE (500 μM). Intracellular levels of cysteine (A) and extracellular levels both of cysteine (B) and CIPE (C) were assessed by HPLC analysis. Each point is the mean (± SEM) of three separate experiments.

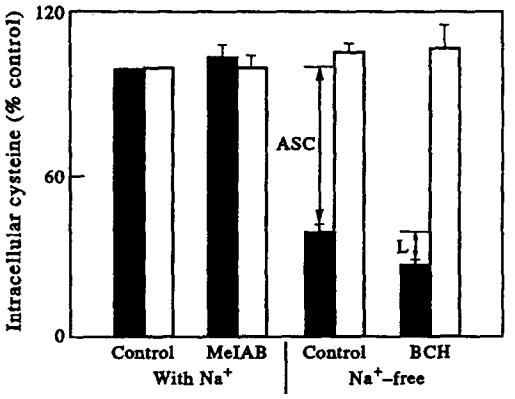

Fig. 7. Effect of inhibitors of the A, ASC and L neutral amino acid systems on the elevation of intracellular cysteine by exogenous cysteine and CIPE. Slices were incubated with cysteine (■) or CIPE (□) (250 μM) for 15 min, with MeIAB (25 mM) to characterize the A system. Slices were also incubated in Na^+-free medium and the decline in the accumulation of exogenous cysteine is characteristic of the ASC system. BCH (10 mM) further reduced the accumulation of cysteine. None of the inhibitors reduced the elevation of cellular cysteine by CIPE. The results are expressed as a percentage of the control elevation of intracellular cysteine by CIPE or cysteine (250 μM) after 15 min (CIPE 1.47 ± 0.07 nmol/mg, cysteine 0.77 ± 0.08 nmol/mg).

Elevation of cellular cysteine by exogenous cysteine and CIPE in the presence of inhibitors of neutral amino acid uptake systems

Several tools were used to block the neutral amino acid uptake systems in order to investigate both the possible role of these systems in the elevation of intracellular cysteine by esters of cysteine and also to determine which of the systems, A, ASC or L, contributes to the accumulation of exogenous cysteine by the lung.

Lung slices incubated with a range of cysteine concentrations (up to 500 μM) produced a linear increase in intracellular cysteine after 15 min (not shown). 2-(Methylamino) isobutyric acid (MeIAB) (25 mM), a specific inhibitor of the type A neutral amino acid uptake system [6, 7], had no significant effect on the intracellular concentrations of cysteine (Fig. 7). Removing the Na^+ from the extracellular medium, characterizing the ASC system [6, 7], substantially reduced the ability of lung slices to sequester cysteine from the extracellular medium (Fig. 7).

BCH (10 mM) in a Na^+-free environment is an inhibitor of the L system [6, 7], and it further reduced the ability of cysteine to elevate cellular levels of the amino acid but only to a small extent (Fig. 7).

CIPE (up to 250 μM) elevated intracellular cysteine levels of slices in a concentration dependent manner and to a greater extent than achieved with cysteine alone (not shown). Coincident exposure of slices to MeIAB, a Na^+-free medium or BCH did not affect the promotion of intracellular cysteine by CIPE (Fig. 7).

DISCUSSION

Inhibition of esterase activity profoundly affected the metabolism of cysteine esters by both rat lung slices and isolated hepatocytes and demonstrated a critical role for esterases in the disposition of the cysteine esters. Esters had longer half lives in the extracellular medium (Figs 3, 4 and 6) and the speed at which they elevated the levels of intracellular cysteine was reduced (Figs 2 and 6). However, esterase inhibition did not greatly increase intracellular levels of the unmetabolized ester.

The esters of cysteine are aliphatic esters and are

probably substrates for the carboxylesterases (Type B-esterase) [19]. The liver has the highest concentration of esterase activity towards simple aliphatic compounds, but the lung also has significant amounts of B-type carboxylesterase activity [20, 21]. A number of carboxylesterases have been identified in mammalian liver both within the cytosol and the endoplasmic reticulum, with a wide spectrum of specificities for substrates and inhibitors [19].

Paraoxon and bis(4-nitrophenyl) phosphate act by an irreversible phosphorylation of the active site of the enzyme and have an inhibitory effect on a variety of carboxylesterases [22]. Although paraoxon has been reported to be a better inhibitor of carboxylesterase activity than bis(4-nitrophenyl) phosphate in isolated enzyme fractions and isolated hepatocytes [22, 23], no differences were observed between these inhibitors in slowing the rate of hydrolysis of CIPE and CCHE by hepatic and pulmonary esterases (Figs 2–4 and 6).

In none of our studies were large amounts of either ester detected intracellularly. Our initial hypothesis was that esterification of cysteine should increase its lipophilicity, so facilitating its cellular accumulation, by obviating the need for cysteine to be accumulated by the neutral amino acid uptake system. Following uptake, we reasoned that the esters should be hydrolysed by intracellular esterases, enhancing cellular cysteine levels, in a manner analogous to the elevation of cellular GSH by esters of GSH [24]. The esters of cysteine elevated the levels of cysteine in lung slices to a greater degree than cysteine alone (Fig. 1) and *in vivo* selectively promoted levels of pulmonary cysteine [14, 15], but without increasing intracellular ester levels. In order to help understand why we were unable to detect unmetabolized esters of cysteine intracellularly, we utilized two inhibitors of esterase activity. These clearly prevented the cleavage of CIPE (Figs 2–4 and 6) but they also reduced the efficiency of the esters to elevate intracellular cysteine (Figs 2 and 6).

We suggest three possible mechanisms by which esters of cysteine may promote cellular cysteine levels: (1) due to their inherent lipophilicity, (2) cysteine generated from the esters extracellularly being accumulated by the neutral amino acid uptake system or (3) possibly by a plasma membrane associated esterase.

Our initial hypothesis (Fig. 8, part 1) was that the increased lipophilicity of the esters relative to cysteine should allow the esters to cross the plasma membrane by diffusion, which would be followed by esterase-mediated hydrolysis. If cellular carboxylesterase activity is inhibited, the levels of intracellular cysteine esters should increase with little or no elevation of cellular cysteine, dependent on the degree of esterase inhibition. The situation should be similar to that observed with paraoxon-pretreated hepatocytes incubated with acetyl salicylic acid, when intracellular levels of the ester and salicylate are increased and decreased, respectively [23]. However, in the present studies, despite high levels of extracellular ester, no elevation in intracellular levels of cysteine ester was observed.

A second possibility (Fig. 8, part 2) suggests that

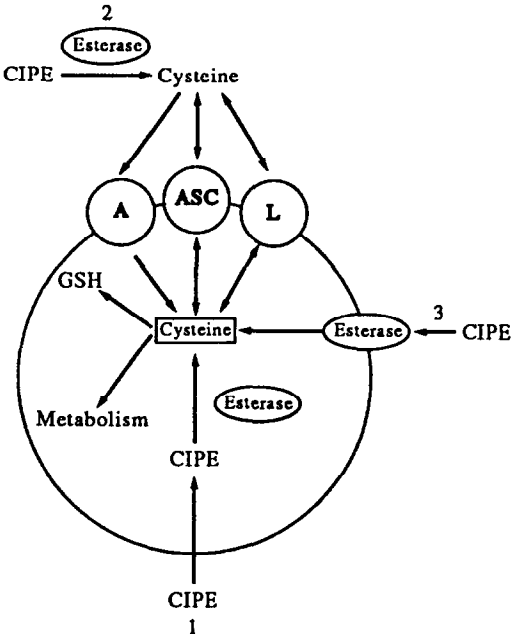

Fig. 8. Possible routes by which CIPE may enter cells. Key: Routes by which CIPE may increase intracellular cysteine; **1**—lipophilicity, **2**—extracellular hydrolysis and accumulation of cysteine via the neutral amino acid uptake systems, **3**—esterase-mediated uptake.

the esters are cleaved in the extracellular medium, releasing cysteine, which will then be taken up by the neutral amino acid uptake systems (A, ASC or L) [6, 7]. In this model, the cysteine esters should only be as effective as cysteine in elevating intracellular cysteine. This suggestion is not supported by the data in Fig. 1. In addition, lung slices incubated in Na^+-free medium are less able to accumulate cysteine whereas the elevation of intracellular cysteine by CIPE was unaffected by Na^+-free medium (Fig. 7).

A third possibility (Fig. 8, part 3) proposes that the esters of cysteine would be cleaved at the plasma membrane, liberating cysteine to the intracellular compartment. Inhibiting esterase activity should both prolong the life span of the ester in the medium and also slow the elevation of intracellular cysteine. The data obtained with both CIPE and CCHE in lung slices and isolated hepatocytes exposed to bis(4-nitrophenyl) phosphate or paraoxon were compatible with this hypothesis. Inhibition of esterase activity reduced the ability of the esters of cysteine to enhance cellular levels of cysteine, especially at 4° (Figs 2 and 6), without a compensatory increase in intracellular CIPE and greatly increased the half life of extracellular esters. We therefore propose that plasma membrane associated esterase activity is involved in the accumulation of esters of cysteine. This is a novel role for esterases in the cell, as the majority of carboxylesterase activity is located in the microsomal and cytosolic fractions [20–22]. However, a specific carboxylesterase, involved in the hydrolysis

of isoprenylated substrates, is found associated with rod outer segment membranes [25].

Lung slices incubated with esters at 4° forced a massive burden of cysteine onto cells after 2 hr (Fig. 2). A similar result is observed when isolated hepatocytes were treated in the same manner (unpublished data). The lower temperature possibly modified the export of cysteine from the intracellular compartment. Lowering the temperature to 20°, retards the efflux of cysteine from kidney cortex slices [26]. We observed a reduction of exogenous cysteine accumulation at 4° (Fig. 5), which suggested that the ASC and L neutral amino acid uptake systems were sensitive to temperature. As the export of cellular cysteine can be mediated by the ASC and L systems, via *trans* stimulation [6, 7], their inhibition at 4° would diminish the efflux of cysteine and force extraordinary levels of intracellular cysteine to build up.

In summary, we observed an important role for esterase activity in the disposition of CIPE and CCHE. Inhibition of esterase activity prolonged the half life of the cysteine esters and reduced their ability to increased intracellular cysteine but without any concomitant elevation of intracellular ester levels. We propose a novel role for plasma membrane bound esterases which mediates the elevation of intracellular cysteine by the esters of cysteine.

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