Glutathione biosynthesis in the isolated perfused rat lung: utilization of extracellular glutathione

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The isolated perfused rat lung catalyzed the biosynthesis of GSH when the sulfur amino acids cysteine or *N*-acetylcysteine, but not methionine, were supplied in the perfusion medium. The lung also had the capacity to utilize extracellular GSH for this purpose. Replenishment of intracellular GSH in perfused lungs from diethylmaleate-treated rats was pronounced even at 25 μM GSH in the perfusion medium. The utilization of extracellular GSH is probably primarily through extracellular break-down and resynthesis rather than direct uptake as indicated by the inhibitory effect of the γ-glutamylcysteine synthetase inhibitor, buthionine sulfoximine and the γ-glutamyl transferase inhibitor, anthglutin. The results indicate that the lung in addition to the kidney may utilize circulating plasma GSH.

GSH *Perfused lung* *Biosynthesis*

1. INTRODUCTION

The biosynthesis of glutathione (GSH) in mammalian liver and kidney has been the subject of extensive study in the past [1]. There exists a considerable intra- and interorgan heterogeneity in the utilization of sulfur amino acid precursors of cysteine for the biosynthesis of GSH. Whereas the liver has the ability to synthesize cysteine from intra-cellular methionine and glycine via the cystathionase pathway [2], neither extra-cellular cystine nor GSH can serve as cysteine precursors in this organ. Alternatively, the kidney utilizes both cystine and GSH for this purpose and appears to lack the cystathionase pathway [5,6].

The kidney is thus the primary organ utilizing circulating plasma GSH [7,8], which is predominantly released from the liver [8,9]. It has been estimated that approx. 2/3 of the breakdown of plasma GSH is due to renal γ-glutamyl-transpeptidase activity [10]. The lung may also contribute to the utilization of plasma GSH as indicated by the finding that isolated lung cells and isolated perfused lung from rat have the ability to utilize extracellular GSH for glutathione conjugation [11].

We examined the ability of the perfused rat lung to utilize different sulfur amino acids for GSH biosynthesis. The mechanism by which extracellular GSH is utilized for this purpose is also discussed.

2. MATERIALS AND METHODS

Glutathione (oxidized and reduced), amino acids, diethylmaleate and bovine serum albumin (fraction V) were all purchased from Sigma (St. Louis, MO). Buthionine sulfoximine was purchased from Chemical Dynamics Corporation (South Plainfield, NJ) and anthglutin (1-γ-L-glutamyl-2-(2-carboxyphenyl)-hydrazine) was the generous gift of Dr M. Tanaka, Sankyo Laboratories, Tokyo, Japan. All other chemicals and reagents were of analytical grade, obtained from local commercial suppliers.

Male Sprague–Dawley rats (150–250 g) were used throughout. In most experiments, animals

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were treated with 0.6 mmol/kg diethylmaleate (DEM) in corn oil (20% w/v) 1 h prior to surgery. The rats were then anaesthetized with pentobarbital and tracheostomized. The chest was opened and the pulmonary artery cannulated. The entire right ventricle and the right atrium together with most of the left ventricle were removed and the left atrium cannulated and secured with a ligature [12]. The lung preparation was dissected free and placed in a negative pressure perfusion apparatus [13]. The lungs were perfused in a recirculating system at a perfusion pressure of 7cmH_2O and a flow rate of 9–10 ml/min with a Krebs-Henseleit buffer (pH 7.4) containing 2% bovine albumin, 5 mM glucose and 25 mM Hepes. The respiratory rate was 70 cycles/min. Some experiments were performed using a single pass perfusion with the same buffer and operating conditions.

Following perfusion, the lungs were weighed and homogenized in perchloric acid (3% final concentration). Samples were centrifuged to remove the precipitated protein and aliquots of the supernatant were derivatized for glutathione determination according to the method of [14]. When the perfusion medium contained GSH or GSH precursors, the lungs were perfused for an extra 15 min with thiol- and disulfide-free buffer in a nonrecirculating manner.

3. RESULTS AND DISCUSSION

Perfused lungs from control rats contained about 1 μM GSH per g wet weight at the beginning of the perfusion. Following perfusion with an amino acid-free medium for 60 min this figure was reduced by 20–30%. Prior treatment of animals with DEM resulted in ~90% depletion of lung GSH. Perfusion of these lungs with an amino acid-free medium failed to increase the GSH content significantly. The inclusion of cysteine in the perfusion medium was shown to support intracellular GSH synthesis with levels returning to ~70% of control values after 60 min perfusion (table 1). It is apparent that the lung does not require additional glycine or glutamate for its GSH biosynthesis and that the intracellular pools of these amino acids are adequate to ensure synthesis, at least for the limited perfusion times used in the present investigation. This also appears to be true for isolated hepatocytes [2], whereas in isolated kidney cells GSH synthesis appears to depend at least partially on the availability of extracellular glycine and glutamate [6].

Table 1

Replenishment of GSH in the perfused rat lung

Conditions	nmol GSH/g tissue (wet wt)
Control	917 ± 112
DEM treated	70 ± 25
+ Cysteine, 1 mM	653 ± 17
+ *N*-Acetylcysteine, 1 mM	509 ± 55
+ Methionine, 1 mM	106 ± 45
+ Cystine, 0.5 mM	248 ± 63
+ GSH, 1 mM	506 ± 43
+ GSH, 2.5 mM	533 ± 19
+ GSSG, 1.25 mM	225 ± 24

Perfusions were run for 60 min. Data are given as means ± SD, $n = 3$–5

N-Acetylcysteine supplied in the perfusion medium was also shown to support GSH synthesis in the lung (table 1), indicating the ability of the rat lung to deacetylate *N*-acetylcysteine to cysteine in this system. However, in the presence of methionine no GSH biosynthesis was observed, indicating that the rat lung lacks one or more of the enzymes involved in the cystathionase pathway.

On the other hand, it seems that the rat lung has the capacity to reduce the disulfide bond in cystine. This has been noted for other cell types and, for instance, kidney cells can utilize cystine as efficiently as cysteine [5,6] for GSH biosynthesis. The lung apparently has only limited reduction capacity, with the efficiency of cystine in supporting GSH synthesis being only 40% that of cysteine. Whether or not cystine is taken up as the disulfide or reduced during the uptake process is not clear. The reduction of cystine may occur via a sequence of reactions catalyzed by thioltransferases, as has been suggested to occur in the kidney [6].

The kidney has a high capacity to degrade glutathione and utilize the amino acids for intracellular GSH synthesis [15]. It is apparent from our experiments that the rat lung also has this ability and may readily utilize exogenous GSH as a source for intracellular GSH. In fact extracellular GSH was as effective as *N*-acetylcysteine in replenishing intracellular GSH. This replenishment was maximal at 1 mM GSH, which may reflect the

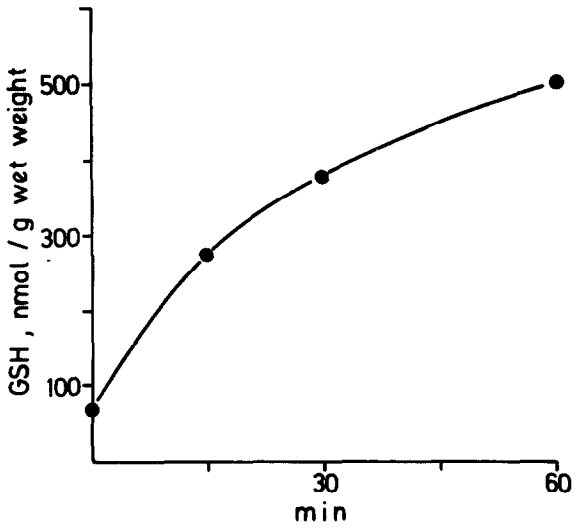

Fig.1. Replenishment of GSH in rat lung from DEM-treated rat perfused with a medium containing 1 mM GSH.

rapid oxidation and disulfide formation in the recirculating system, and the rate of synthesis was ~10 nmol GSH/g per min (fig.1).

The majority of the perfusions were done in a recirculating manner with rather high concentrations of thiols. This was more economical than single-pass perfusions with low substrate concentrations - the high thiol levels being necessary due to disulfide formation. However, in order to show that this phenomenon of GSH uptake and resynthesis by the lung is relevant at physiological concentrations, a small amount of single pass perfusions were performed with various concentrations of GSH and with 100 μM cysteine and *N*-acetylcysteine, for comparison (table 2). It can be seen that with single-pass perfusion, which more closely mimics the physiological situation, the lower concentration of cysteine was almost as effective in supporting GSH resynthesis as was 1 mM cysteine in the recirculating system (tables 1,2). *N*-Acetylcysteine at 100 μM produced resynthesis of up to 25% of the intra-cellular GSH in 1 h (table 2). Single pass perfusion with 25–1000 μM GSH resulted in various rates of resynthesis of intracellular GSH and this was saturated at 200 μM extracellular GSH (table 2) (approx. 9 nmol GSH resynthesized/min). The concentration of GSH in rat hepatic vein has been found to be 21 μM [16] and at 25 μM GSH in the single-pass perfusion the

Table 2

Replenishment of GSH in the lung from DEM-treated rats using single-pass perfusion

Conditions	nmol GSH/g tissue (wet wt)
+ Cysteine, 100 μM	608
+ *N*-Acetylcysteine, 100 μM	228
+ GSH, 25 μM	199
+ GSH, 50 μM	257
+ GSH, 200 μM	607
+ GSH, 1 mM	538

Perfusions were run for 60 min. Results represent duplicate determinations from one experiment in each case

observed rate of intracellular GSH replenishment was approx. 2.15 nmol/min (table 2). It is likely that, had the perfusion period been longer, it may have been possible to observe complete replenishment of intracellular GSH from 25 μM extracellular GSH.

Results of perfusion experiments using the γ-glutamyl-transferase (γ-gt) inhibitor anthglutin [17] and the γ-glutamyl-cysteine synthetase inhibitor buthionine sulphoximine (BSO) [18], indicate that the utilization of extracellular GSH is largely dependent upon breakdown and re-synthesis as opposed to direct uptake. In the presence of anthglutin, the GSH-dependent replenishment of intracellular GSH was inhibited by greater than 50% (table 3), resulting presumably from the inhibition of the breakdown of extracellular GSH so that the availability of the precursor amino acid becomes rate limiting. Alternatively, this observation may be explained by anthglutin inhibition of γ-gt-mediated uptake of GSH.

Results obtained from experiments using BSO favor the idea of γ-gt-mediated breakdown of extracellular GSH followed by resynthesis intracellularly. Total inhibition of GSH replenishment by extracellular GSH and by other sulfur amino acids was achieved when BSO was included in the perfusion medium. BSO is regarded as a relatively specific inhibitor of γ-glutamylcysteine synthetase, binding to the active site of the enzyme, and when administered in vivo has been shown to decrease GSH in most tissues investigated [19].

However, as indicated by findings of authors in [20], BSO may have other effects. They showed

Table 3

Replenishment of GSH in the perfused lung from DEM treated rats

Conditions	nmol GSH/g tissue (wet wt)
DEM treated	70 ± 25
+ Cysteine, 1 mM	653 ± 17
+ Cysteine + BSO, 0.5 mM	109 ± 48
+ GSH, 1 mM	506 ± 43
+ GSH + BSO, 0.5 mM	48 ± 39
+ GSH, 1 mM + anthglutin, 0.2 mM	236 ± 116

Perfusions were run for 60 min. Data are given as means ± SD, n = 3–5

that the uptake of liposome entrapped GSH into liver and spleen was inhibited by BSO. Thus, one cannot exclude the possibility that BSO interferes with the uptake of GSH into the lung, even though it seems more likely that the effect of BSO is through inhibition of the resynthesis of GSH.

In conclusion, it has been demonstrated that the isolated perfused rat lung synthesizes GSH when the sulfur amino acids cysteine and *N*-acetylcysteine are supplied in the perfusion medium. The lung also has the capacity to utilize extracellular GSH, probably through an extracellular breakdown and resynthesis mechanism. This is in support of previous findings where the conjugation of fluorodinitrobenzene in the perfused lung was increased in the presence of GSH in the perfusion medium [11].

There is a considerable interorgan transport of glutathione. The circulating plasma levels of glutathione are quite high and found predominantly in the reduced form [8]. Liver appears to be the organ responsible for the 'export' of most of the GSH found in plasma, whereas the kidney utilizes plasma glutathione efficiently, since this organ has a high γ-gt activity. About 2/3 of the plasma glutathione has been estimated to be utilized by the kidney [10]. Our results indicate that the lung may utilize the major part of the remaining plasma glutathione.

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