Inhibition of glutathione efflux in the recirculating rat liver perfusion by cysteine but not by oxothiazolidine carboxylate, an intracellular cysteine precursor

Eltjo J. Glazenburg, Jan E. Bruggink, Katja Wolters-Keulemans and Gerard J. Mulder*

Department of Pharmacology, State University of Groningen, Bloemsingel 1, 7913 BZ Groningen, and **Division of Toxicology, Center for Bio-Pharmaceutical Sciences, University of Leiden. PO Box 9503, 2300 RA Leiden, The Netherlands*

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In the recirculating rat liver perfusion a continuous release of glutathione into the perfusion medium is observed. Addition of L-cysteine to the perfusion medium immediately arrested this glutathione efflex. The cysteine precursor oxothiazolidine carboxylate did not block the glutathione efflux in spite of the fact that it generated more L-cysteine inside the liver cells than L-cysteine itself; L-cysteine is rapidly oxidized to cystine, that is no longer taken up by the liver. The results suggest that the inhibition of glutathione efflux results from the presence of cystine in the perfusion medium.

Cysteine *Cystine* *Glutathione* *Liver perfusion* *Oxothiazolidine carboxylate* *Sulfate*

1. INTRODUCTION

Rat liver hepatocytes continuously release glutathione, as observed in both the single pass perfused liver [1,2] and isolated hepatocytes [3,4]. It is mainly released as reduced glutathione (GSH), but this rapidly oxidizes in air to oxidized glutathione (GSSG). Under situations of oxidative stress the liver releases mainly oxidized GSSG [5,6]. The mechanisms of these glutathione effluxes remain unelucidated.

It was reported in [4] that glutathione efflux from isolated rat hepatocytes could be inhibited by methionine and some analogs; several other amino acids were not effective. This inhibition was not due to inhibition of GSH synthesis, nor was it dependent on Na^+, synthesis of cysteine or ATP generation. Here we show that cysteine has a similar effect, while L-2-oxothiazolidine-4-carboxylate (OTC), an intracellular L-cysteine precursor [7], has no such effect.

* To whom correspondence should be addressed

2. MATERIALS AND METHODS

L-[^{35}S]Cysteine (spec. act. 790 Ci/mmol) was purchased from NEN, Dreieich. OTC was synthesized as in [8].

For the recirculating, isolated rat liver perfusion system, male Wistar rats (260–280 g body wt) were used that had free access to food and water. The liver weight was 9.7 ± 0.3 g. The perfusion system has been extensively described [9]. The erythrocyte-free perfusion medium consisted of Krebs-bicarbonate buffer, pH 7.4, in which $MgSO_4$ was replaced by $MgCl_2$, so that initially no sulfate was present; 1% (w/v) bovine serum albumin was added. Oxygenation was provided by carbogen gas, and the liver flow was 40 ml/min. After a pre-perfusion period of 30 min (employing 100 ml perfusion medium), 1 ml of a freshly prepared solution of either 100 mM L-[^{35}S]cysteine, or 100 mM OTC was added. Samples of perfusion medium and bile were taken during the perfusions which lasted for 2–4 h.

Inorganic sulfate was determined as in [10].

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Reduced L-cysteine was determined according to [11]. Total cysteine was measured similarly after reduction of disulfides with dithiothreitol as in [12]. GSH was determined fluorimetrically [13]; total glutathione was measured similarly after reduction with dithiothreitol.

3. RESULTS

In the recirculating rat liver perfusion a steady increase in the glutathione concentration in the perfusion medium was observed, due to efflux of glutathione from the liver (fig.1). In this recirculating, well oxygenated system glutathione was virtually exclusively present as GSSG. Glutathione accumulated to approx. 550 μM after 3 h. When, after 30 min pre-perfusion, L-cysteine was added, there was a rapid drop in glutathione concentration, after which it increased only very slowly for the next 2 h, indicating that the efflux of glutathione from the liver was greatly reduced (fig.1). Reduced L-cysteine disappeared very rapidly from the perfusion medium, mainly due to oxidation to cystine. The cystine formed was only slowly taken up by the liver as determined both chemically and radiochemically (fig.2). Only reduced cysteine, present during the first few minutes, seemed to be taken up rapidly by the liver; however, relatively little became available inside the hepatocytes, since no increase of inorganic sulfate in the perfusion medium was observed (fig.3). Since the effect of cyst(e)ine persisted during the whole perfusion we wanted to know whether this was an effect of cysteine (inside the hepatocyte) or of cystine (in the perfusion

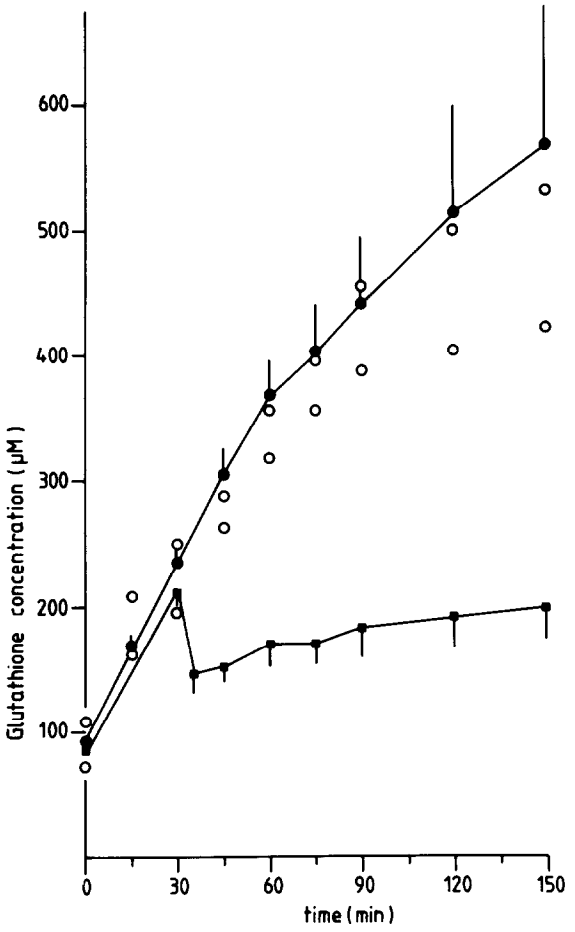

Fig.1. Efflux of glutathione in the isolated recirculating rat liver perfusion. The livers were perfused for 3 h. After a pre-perfusion period of 30 min, either L-cysteine (■—■; n = 3) or OTC (●—●; n = 4) were added to a final concentration of 1 mM. Two controls (○) in which only solvent was added are also shown. Mean ± SE is shown.

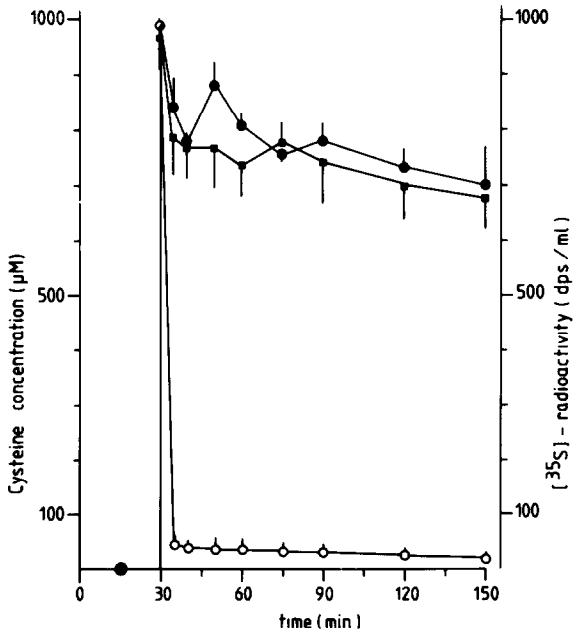

Fig.2. Disappearance of L-cysteine from the perfusion medium. After a pre-perfusion of 30 min, L-[^{35}S]cysteine was added to a final concentration of 1 mM. The concentration of L-cysteine (○—○), total cyst(e)ine (mainly cystine; ●) as well as total ^{35}S radioactivity (■) are given.

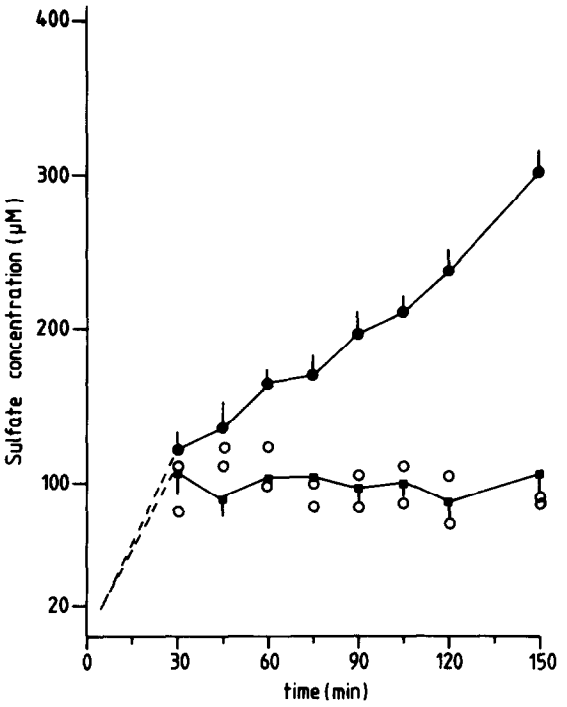

Fig.3. Release of inorganic sulfate in the perfusion medium. The concentration of inorganic sulfate in the perfusion medium, which contained initially no sulfate, is shown. During the pre-perfusion inorganic sulfate leaks from the liver to equilibrium. After 30 min, either L-cysteine (■), OTC (●) or solvent (○) were added.

medium). Therefore, we have also determined the effect of OTC, which is converted to L-cysteine inside liver cells. Clearly, OTC had no effect on the glutathione efflux (fig.1), although it increased the concentration of inorganic sulfate in the perfusion medium considerably, in contrast to added L-cysteine.

The concentration of glutathione was determined in livers after the perfusion. The glutathione content of livers to which L-cysteine had been added was (after 2.5 h perfusion) 3.10 ± 0.10 μmol/g liver, while in the OTC perfusions the level was 0.72 ± 0.05; in both cases approx. 90% was GSH. In one control perfusion the level was determined at 1.12 μmol/g liver, of which again 90% was GSH.

4. DISCUSSION

Our results show that either cysteine or cystine inhibits the efflux of glutathione from the liver, an effect that appears to be similar to that observed by authors in [4] for methionine. They found that the effect of methionine did not require its conversion to cysteine, but they did not test cyst(e)ine itself. Now fig.2 shows that within a few minutes L-cysteine in the perfusion medium had almost completely been oxidized to cysteine, while a part was also taken up by the liver. The fact that the inhibition persisted after L-cysteine had disappeared suggested that either L-cysteine taken up was responsible for the effect, or that cysteine inhibited the efflux, since its concentration hardly decreased during the perfusion. However, because OTC had no effect on glutathione efflux, the inhibition is most likely mediated by cystine. In fact, the production of inorganic sulfate only after OTC indicates that more cysteine became available in the cell after OTC than after L-cysteine itself. Since little cystine is present inside the hepatocyte [14] the effect of cystine may be effected from the outside of the hepatocytes. In agreement with the data in [4] we found much higher GSH levels in the L-cysteine perfused livers than in the OTC livers again in spite of the fact that OTC seemed to yield more intracellular cysteine: due to a decreased efflux, a higher residual GSH inside the liver at the end of the perfusion resulted.

The fact that we find GSSG rather than GSH in the perfusion medium most likely is due to the rapid oxidation of GSH in the well oxygenated recirculating perfusion medium.

Another finding is that cysteine seems to be much faster taken up by the liver than cystine, an observation that seems not to have been reported before [14]; this may be related to the relatively low levels of γ-glutamyltranspeptidase in the liver [15]. In recirculating perfusions, therefore, L-cysteine cannot be added to increase the availability of this amino acid, since it will be rapidly oxidized, unless an antioxidant is added as well.

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