REDUCED AND OXIDIZED GLUTATHIONE EFFLUX FROM LIVER

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1. Introduction

Recently, evidence for interorgan relationships in the turnover of glutathione has accumulated [1–4], and a steady state plasma glutathione concentration of 5 μM measured in rat plasma [5]. Thus, the concept is emerging of the kidney as a major site of glutathione degradation to the constituent amino acids, whereas other organs, e.g., the liver, synthesize their own glutathione pool and, furthermore, contribute to the plasma pool in an as yet ill-defined manner.

Several studies have shown that cells release glutathione disulfide (GSSG) when exposed to 'oxidative stress', most probably as a consequence of an increased intracellular concentration of GSSG due to the action of glutathione peroxidase, e.g., in lens cells [6], erythrocytes [7–9] and liver cells [10–13]. However, the release of glutathione from cells in the absence of experimentally-imposed oxidative conditions has been less extensively studied. Isolated perfused rat liver releases GSSG at a small but significant rate of 1–2 nmol GSSG/min/g liver (wet wt), as was found in our earlier studies [10,14], and total glutathione, GSSG + GSH, was found to be released at rates between 6–12 nmol GSH equivalents/min/g perfused liver at 30°C [12,13], or about 6 nmol/min/g (10^8 cells) isolated hepatocytes [15].

Results of separate measurement of GSH and GSSG released from the perfused liver are presented here, indicating that glutathione efflux occurs largely as GSH, whereas the extra release of glutathione elicited by an acceleration of the glutathione peroxidase reaction occurs as GSSG. Further, it is estimated that the observed rates are compatible with the in vivo turnover of glutathione.

2. Methods

Livers from male Wistar rats of 140–180 g body wt, fed on stock diet (Altromin), were perfused at 37°C as in [11] except that L-lactate/pyruvate was 2.1 mM/0.3 mM. Perfusate flow rate was 4 ml/min/g liver. Effluent perfusate was assayed directly for lactate dehydrogenase activity, for glutathione disulfide (GSSG) by following the decrease of NADPH absorbance after addition of glutathione reductase [11], for reduced glutathione (GSH) by following the formation of *S*-lactoyl glutathione from methylglyoxal, catalyzed by glyoxalase I [16,16a], and for GSH+GSSG in the catalytic assay using glutathione reductase and 5,5′-dithiobis-(2-nitrobenzoic acid) [5,12,17]. In view of the low concentrations in the samples, GSSG and GSH were determined by the dual-wavelength method at 340–380 nm and 240–270 nm, respectively, in a Shimadzu Model UV 300 spectrophotometer, whereas the GSH–GSSG determination was carried out at 405 nm in an Eppendorf model 6114 spectrophotometer. Calibrations were carried out by the principle of internal standards, and recoveries at the low substrate concentrations were ascertained.

3. Results and discussion

3.1. *Rates of glutathione release from perfused rat liver*

The release of glutathione into the effluent perfusate was measured by three different methods (table 1). The rate of release of glutathione disulfide was found to be 1.0 nmol/min/g liver, in agreement

Table 1
Release of glutathione and of lactate dehydrogenase from hemoglobin-free perfused rat liver

Parameter	Rate of release from liver	
	Control	*t*-Butyl hydroperoxide (0.5 μmol/min/g)
GSH (nmol/min/g)	11.8 ± 0.4 (8)	11.2
GSSG	1.0 ± 0.1 (8)	18.7
GSH + GSSG, in GSH equiv.	13.8	48.8
GSH+GSSG, in GSH equiv. (determined with catalytic assay)	14.5 ± 0.8 (8)	45.2
Lactate dehydrogenase activity (mU/min/g)	11.2 ± 1.6 (5)	12.0

Perfusate flow, 4 ml/min/g liver wet wt. Temp. 37°C. Data are expressed as means ± SEM (no. different perfusions in parentheses). Values were determined in triplicate in each experiment (three for addition of *t*-butyl hydroperoxide)

with our earlier measurements [10,11,14]. The rate of release of glutathione in the reduced form was about 10-fold higher, 11.8 nmol GSH/min/g liver, so the total amounts to 13.8 nmol/min/g as expressed in GSH equivalents. The value found with the third method, using Ellman's reagent and glutathione reductase, is 14.5 nmol/min/g, again expressed in GSH equivalents.

The total GSH content of the liver is 5.28 μmol/g [10], so that the observed rate of release corresponds to 0.3% total glutathione/min. Thus, the observed release of glutathione is compatible with the half-life of glutathione of about 4 h as calculated from isotope measurements [18,19]. It is possible, therefore, that the observed release of glutathione from the perfused liver reflects a physiological process rather than an experimentally-induced leakage. This is supported by the low rate of release of lactate dehydrogenase, a cytosol marker, into the effluent perfusate. The rate of 11.2 mU/min/g (table 1) corresponds to 0.004% total lactate dehydrogenase content, i.e., to a rate of release 2 orders of magnitude lower than that of glutathione.

3.2. *Alterations in rate of release and redox state of glutathione*

When the rat was pretreated with maleic acid diethylester (0.6 mmol/kg intraperitoneally 1 h before perfusion) to deplete glutathione [20], there was a very low release of 1.8 nmol/min/g as measured in the catalytic assay for GSH+GSSG, whereas neither GSH nor GSSG were detectable in the perfusates.

An anoxic interval of up to 20 min did not cause changes in the rates of release.

In agreement with earlier observations, the extra release of glutathione elicited by the addition of *t*-butyl hydroperoxide to the influent perfusate occurred in the form of GSSG [10,11], as shown in table 1, whereas *t*-butanol exhibited no effect.

Glutathione release occurred at lower rates when the temperature was lowered, thus explaining the somewhat lower values observed at 30°C [12,13] as compared to 37°C.

3.3. *Relation of glutathione efflux from perfused liver to plasma glutathione level and renal γ-glutamyl-transpeptidase*

The physiological significance of the observed glutathione efflux from the isolated perfused liver remains to be evaluated. With a liver weight of 4.35 ± 0.11 (n = 11) g/100 g/body wt, the observed rate of glutathione efflux of 14 nmol/min/g liver (table 1) would correspond to a rate of about 60 nmol GSH/min/100 g rat if the efflux occurs at a similar rate in vivo. As the kidney has been identified as a major site of degradation of extracellular glutathione

[2–4] and the γ-glutamyltranspeptidase localized in the brush border membrane has been shown to efficiently degrade glutathione derivatives like *S*-methyl GSH and bromsulfalein-GSH [21], it would be of interest to estimate the approximate capacity of renal plasma glutathione degradation in the intact organism. A preliminary calculation with the plasma glutathione concentration of 5 μM in blood samples obtained by heart puncture [5] showed that this capacity is lower than the above-mentioned rate, due to a limitation by the renal plasma flow.

Thus, further detailed comparisons of the intact organism and the perfused organ will be of interest to define the role of glutathione efflux in glutathione turnover and to elucidate the interorgan relationships in this process.

Finally, it may be noted that the redox state of the glutathione released from the perfused organ is shifted only slightly towards oxidation as compared to the in situ value of 0.051 for GSSG/GSH as obtained in freeze-clamped liver samples [22].

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