ASSESSMENT OF THE KIDNEY FUNCTION IN MAINTENANCE OF PLASMA GLUTATHIONE CONCENTRATION AND REDOX STATE IN ANAESTHETIZED RATS

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1. Introduction

Substantial evidence has been provided for a role of the kidney in the metabolic turnover of extracellular glutathione [1–12]. Glutathione has been found in the extracellular space at 3–5 μM in the arterial plasma of the rat [7,13] and in human venous plasma [14]. In studies of the fate of extracellular glutathione the degradation has been proposed to occur mainly on the luminal surface of the renal brush-border membrane and that the γ-glutamyl transpeptidase is a glutathionase acting on extracellular glutathione. However, a quantitative assessment of this function of the kidney enzyme in the intact animal was not performed. In order to provide more information on this hypothesis, we measured glutathione uptake by the kidney in the anaesthetized rat [7]. Interestingly, renal glutathione extraction was ~90%. In view of the renal filtration fraction of ~30% this indicated that, in addition to the intratubular degradation, there must be a further uptake mechanism for glutathione by the kidney, in accordance with findings with the isolated perfused rabbit kidney infused with 1.6 mM GSH [4].

Here we have assessed the capacity of renal glutathione uptake in the anaesthetized rat in relation to that of the extrarenal tissue. For this purpose GSH or GSSG were infused into the jugular vein. The glutathione plasma concentration and the redox state were studied by nephrectomizing the animals, and the net rate of glutathione release from tissue into plasma was estimated on the basis of steady state calculations.

Prompted by the report [10] on renal GSH oxidase activity [2], the effect of a thiol reductant, dithioerythritol, on renal glutathione uptake was investigated.

Two interesting new results turned out:

(i) Plasma glutathione is present to ≥50% in the oxidized disulfide form, similar to that found in the rat bile, 35% [15], whereas intracellular glutathione has been shown present only at ~2–12% oxidized.

(ii) The kidney was estimated to account for ~50% of the net plasma glutathione turnover which is of the order of 10–20 nmol GSH equiv. min^{-1}. 100 g body wt^{-1}.

Abbreviations: GSH, reduced glutathione; GSSG, glutathione disulfide

When the term 'glutathione' as such is used, no inference is made as to the redox state, but quantitative statements refer to 'GSH equivalents'. Mixed disulfides are not considered here

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2. Materials and methods

2.1. *Preparation of animals*

Experiments were performed on male Sprague-Dawley rats (300–420 g body wt) fed with a stock diet (Altromin Standard Diät). Following i.p. injection of sodium 5-ethyl-5(1-methyl-propyl)-2-thio-

barbiturate (100 mg/kg body wt), the animals were tracheotomized, and the right external jugular vein catheterized for the infusions. Blood pressure was monitored in the left femoral artery.

In renal glutathione extraction experiments, a catheter was inserted into the fundus of the bladder. Inulin clearance measurement of the right kidney served as control for the experimental left kidney. The left kidney was exposed and immobilized in a lucite cup. The left ureter was then cannulated.

In nephrectomy experiments the renal pedicles were exposed and ligated and then the kidneys removed.

2.2. *Experimental protocol*

Jugular vein infusion rates were 1.5 ml . h^{-1} . 100 g body wt^{-1}. Infusions were isotonic saline during renal surgery, and after completion of surgery, in addition 2.5 g polyfructosan (Inutest, Laevosan, Linz)/100 ml and, as indicated in the tables, GSH or GSSG with and without dithioerythritol at 16–160 mM to give the infusion rates noted in tables and figures.

After an equilibration period of $\geqslant$ 30 min, heparin (750 US-E/100 g body wt) was injected. A 100 μl sample of blood was taken from the femoral artery, and urine was collected for each kidney for periods of $\geqslant$ 30 min. Over periods of 5 min, further blood samples were taken: arterial, renal venous and again arterial, each ~0.7 ml.

2.3. *Analyses*

The renal plasma flow and the glomerular filtration fraction were determined by standard procedures as in [16]. Glutathione assays were performed in freshly neutralized perchloric acid extracts of plasma samples for GSSG and for total glutathione, i.e., GSH plus 2 GSSG, as in [13,15,17]. In the latter assay, standardized glutathione was added to each sample since in neutralized extracts the reaction velocity obtained by standard addition was ~2/3rds of comparable blank extracts. Thus, by internal standardization the known partial inhibition of yeast glutathione reductase by biological samples [17a] was taken into account in the catalytic assay [13]. Recovery of added GSH and GSSG was 90–110% within the range of concentrations measured in this study.

3. Results and discussion

3.1. *Glutathione redox state in arterial and renal venous plasma*

Glutathione concentrations in the arterial plasma were found to be 3.1 ± 0.4 μM (n = 7) as shown in table 1, and in a separate group, 3.86 ± 0.37 μM (n=8). In the latter group, GSSG was also determined; its value was 1.03 ± 0.26 μM. Thus, the plasma GSSG concentration accounted for 53% of the total glutathione in plasma. This value may represent a lower limit because GSH is present at 1000-fold excess in the erythrocyte so that even slight (and undetectable by hemoglobin measurement) hemolysis could introduce an error.

In further experiments, the plasma glutathione concentration was raised by intravenous infusion. As shown in table 1, the glutathione concentration was increased in the arterial plasma to 0.63 mM with a GSH infusion of 1.6 μmol . min^{-1} . 100 g body wt^{-1} and to 0.55 mM with a GSSG infusion of 0.8 μmol . min^{-1}. 100 g body wt^{-1}.

The maintenance of the glutathione redox state in plasma is effective, as is revealed by the observation of 74% GSSG of total glutathione in arterial plasma when GSH is infused so that the glutathione level is increased 200-fold above the physiological level (table 1). Further, even when dithioerythritol is infused together with GSH, there is a substantial percentage of GSSG (~ 20%) in arterial plasma. In the renal venous plasma the concentrations rose from 0.4 μM in the controls to 0.2 mM with the infusion of GSH or GSSG. The plasma GSSG concentration accounted for > 70% in these experiments.

Thus, glutathione is present in plasma as the disulfide in a much higher percentage than intracellular glutathione; GSSG was found to be 4–12% in erythrocyte [18], liver [19,20], heart [21], eye lens [22] and aortic tissue [23]. Interestingly, GSSG in rat bile, another extracellular fluid, was found to be high, too, at 35% [15].

Table 2 shows that the glutathione redox state in plasma remains practically unaltered after removal of the two kidneys. Thus, the extracellular glutathione redox state can also be controlled effectively by extrarenal systems, most likely by glutathione oxidases in other epithelial organs [12].

Table 1
Glutathione concentration in arterial and renal venous plasma of the rat and renal plasma flow as well as filtration fraction during infusion of glutathione into the jugular vein

Infusion (μmol . min^{-1} . 100 g body wt^{-1})	Total glutathione plasma conc. (μM GSH equiv.)		% Extraction of renal load	GSSG plasma conc. (μM)		% Extraction of renal load	Renal plasma flow (ml . min^{-1} . g kidney^{-1})	Filtration fraction (%)
	Arterial	Renal venous		Arterial	Renal venous			
None	3.1 ± 0.4[a]	0.39 ± 0.08[a]	88 ± 3	–[b]	n.d.	–	3.04 ± 0.41	26 ± 4
GSH (1.6)	625 ± 34	211 ± 27	66 ± 3	232	89	62	3.28 ± 0.40	32 ± 1
GSSG (0.8)	550	226	59	324	113	65	3.24 ± 0.39	35 ± 1
GSH (1.6) plus dithioerythritol (0.8)	942 ± 75	472 ± 43	49 ± 5	89 ± 5	31 ± 5	64 ± 7	2.65 ± 0.15	42 ± 2
GSSG (0.8) plus dithioerythritol (0.8)	749 ± 33	377 ± 21	50 ± 2	243 ± 20	98 ± 12	57 ± 6	3.09 ± 0.48	37 ± 3
Dithioerythritol (0.8)	6.6 ± 1.7	4.9 ± 1.4	26	–	–	–	–	–

[a] From [7]
[b] See data in section 3.1

The experimental protocol is as in section 2.2. Data are means ± SEM (n = 3–7 different experiments) or means of 2 separate experiments (no SEM); n.d., not detectable: below detection limit

Table 2
Plasma glutathione concentration and redox state following nephrectomy in rats

Time after nephrectomy (min)	Total glutathione (μM GSH equiv.)	GSSG (μM)	Oxidized (%)
30	8.7 ± 1.5 (3)	3.3 ± 0.7 (3)	76
60	10.8 ± 2.7 (3)	3.5 ± 0.6 (3)	65
90	7.5 ± 0.6 (3)	2.8 ± 0.5 (3)	74
120	15.0 ± 0.9 (5)	4.7 ± 0.4 (5)	62

Data are given as means ± SEM, no. different animals in parentheses. The 'percentage oxidized' is calculated by referring the GSSG concentration (2×) to total glutathione. Control glutathione concentration is 3–4 μM (table 1)

3.2. *Renal glutathione extraction*

As shown in table 1, at an arterial glutathione plasma concentration of 3.1 ± 0.4 μM the renal venous plasma glutathione concentration was 0.39 ± 0.08 μM. Thus, 88% of the renal glutathione plasma load was extracted by the kidney. Only 26% of this load had been filtered in the glomeruli (table 1). As indicated in the Introduction, in contrast to earlier conclusions [5,12], renal glutathione uptake cannot, therefore, be explained solely on the basis of γ-glutamyl transpeptidase activity of the tubular brush border membrane which has been elegantly identified by microperfusion of cortical nephrons [6] and by preparative techniques [8]. Rather, an additional glutathione uptake or degradation mechanism of basolateral localization must be operative.

That the renal glutathione extraction capacity is very high is shown in table 1 and fig.1: 60–70% extraction is observed even when the arterial glutathione concentration is raised to 200-fold the control value by infusion of GSH or GSSG. At these plasma concentrations, it can be calculated that the renal extraction accounts almost quantitatively for all glutathione infused. Thus, at an increased influx of glutathione into the plasma, the renal extraction mechanisms determine the steady state glutathione plasma level.

However, at low plasma concentrations, an effective extrarenal capacity for glutathione extraction may also exist. As shown in table 2, after nephrectomy a steady state plasma concentration is maintained for ≥2 h, with the level ~4-fold higher than in control.

The kidney extracted glutathione from plasma independent of its state of oxidation, as is concluded from the similar extraction observed with infusions of GSH or GSSG with and without dithioerythritol (table 1, fig.1). This may be due either to the effective glutathione oxidase to maintain the intrarenal glutathione plasma redox state, or to a non-specificity of the degradation reaction with respect to GSH and GSSG. Purified γ-glutamyl transpeptidase was shown to react with both GSH and GSSG [8].

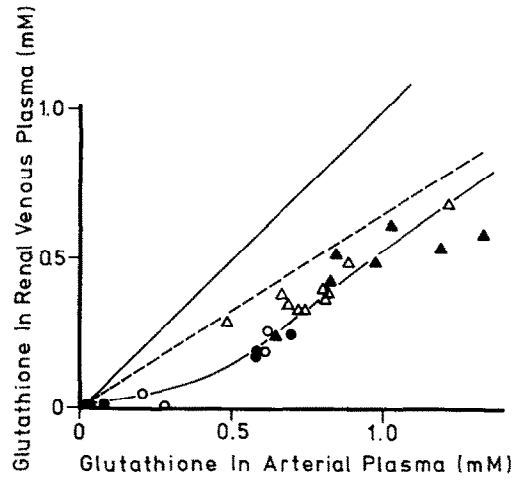

Fig.1(A). Glutathione concentration in arterial and renal venous plasma during infusions of GSH (full symbols), GSSG (open symbols) together with (triangles) and without (circles) dithioerythritol. Infusion rates for GSH or GSSG were varied between experiments to establish varying arterial plasma concentrations whereas dithioerythritol infusions were performed at 0.8 μmol . min^{-1} . 100 g body wt^{-1} (compare table 1). The theoretical lines were drawn to indicate no renal extraction (solid line at 45°) and renal extraction on the basis of glomerular filtration of 35% of renal plasma flow (dashed line).

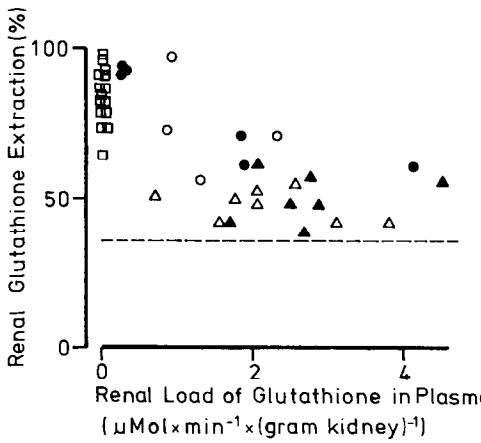

Fig.1(B). Relationship between renal glutathione extraction and renal glutathione load in plasma. Symbols are as in fig.1(A). In addition, the values of control experiments (no infusions) are also shown (rectangular symbols). The dashed line represents the renal extraction of glutathione due to glomerular filtration assuming free solution of glutathione in plasma.

The data presented in tables 1 and 2 allow an estimation of the net rate of glutathione release from the tissues into plasma, if it is assumed that the glutathione release (GSH influx) is equal in the control state and after nephrectomy.

With:

$$\text{GSH influx}_{\text{contr.}} = \text{GSH}_{\text{contr.}} (\text{renal plasma flow} \times \text{renal extraction} + \text{cardiac plasma output} \times \text{extrarenal extraction}) \quad (1)$$

$$\text{GSH influx}_{\text{nephr.}} = \text{GSH}_{\text{nephr.}} (\text{cardiac plasma output} \times \text{extrarenal extraction}) \quad (2)$$

where $\text{GSH}_{\text{contr.}}$ and $\text{GSH}_{\text{nephr.}}$ refer to the respective plasma glutathione concentrations and with:

$$\text{Cardiac plasma output} = 4 \times \text{renal plasma flow} \quad (3)$$

the extrarenal extraction can be expressed in terms of the two GSH steady state concentrations and renal extraction, assuming that the extrarenal extraction (%) is unchanged by nephrectomy. Thus, eq. (2) simplifies to terms of measured quantities:

$$\text{GSH influx} = \frac{\text{GSH}_{\text{nephr.}} \times \text{GSH}_{\text{contr.}} \times \text{renal plasma flow} \times \text{renal extraction}}{\text{GSH}_{\text{nephr.}} - \text{GSH}_{\text{contr.}}} \quad (4)$$

Using the data of tables 1 and 2, glutathione release into plasma is calculated according to eq. (4) to be 10–20 nmol . min^{-1} . 100 g body wt^{-1}. This value is lower than what can be estimated from the GSH efflux from isolated perfused liver, amounting to 12 nmol . min^{-1} . g $liver^{-1}$ [17], or with 4.35 g liver/100 g body wt, to 52 nmol . min^{-1} . 100 g body wt^{-1}.

Further, the data of tables 1 and 2 permit estimation of the relation of the extrarenal to the renal extraction, showing that ≥50% of the glutathione influx from the body tissues is metabolized by the kidney, in agreement with the observation [5] that ophthalmic acid degradation is substantially slowed when the renal arteries are clamped.

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