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DIRECT EVIDENCE FOR INTER-ORGAN TRANSPORT OF GLUTATHIONE AND THAT THE NON-FILTRATION RENAL MECHANISM FOR GLUTATHIONE UTILIZATION INVOLVES γ-GLUTAMYL TRANSPEPTIDASE

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Summary: A direct examination of the inter-organ cycle of glutathione metabolism was made by determining glutathione levels in plasma obtained from various blood vessels of the rat. High levels of GSH were found in hepatic vein plasma, relative to arterial and systemic venous levels, reflecting translocation of GSH from the liver to the plasma. Renal vein plasma has a level that is 20% of arterial plasma indicating that the kidney removes glutathione from plasma not only by glomerular filtration (which can account for 20-30% of the glutathione removed), but also by a non-filtration mechanism. Inhibitors of γ-glutamyl transpeptidase decrease the fraction of glutathione removed by the kidney to a value approaching that filtered, indicating that the non-filtration mechanism involves γ-glutamyl transpeptidase.

Introduction: Previous studies showed that the total glutathione (GSH + GSSG) level in arterial blood plasma of unanesthetized rats is about 25 μM GSH equivalents, and that about 85% of the total glutathione is in the form of GSH (1,2). Recent research has shown that the metabolism of glutathione follows intra-organ and inter-organ cycles (3-6). Thus, GSH synthesized in the liver (and probably other organs) is translocated to blood plasma. Plasma glutathione is removed mainly (~67% (6,7)) by the kidney, which has high γ-glutamyl transpeptidase activity. Renal transpeptidase also metabolizes glutathione translocated from renal cells (intra-organ cycle) (3,5).

We sought here direct evidence for inter-organ transport of glutathione by examining the glutathione levels of blood plasma obtained from different blood vessels. Sies and collaborators (7,8), reported that glutathione level in renal venous plasma is about 10% of that of arterial plasma, but these observations require further scrutiny be-

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cause (a) the values of arterial plasma total glutathione found in these studies (7,8) were extremely low (about 3 μM), and (b) a large fraction of the glutathione was found to be in the form of GSSG; other investigations (1,2) led to substantially different results as stated above.

Experimental Procedures: Male Sprague-Dawley rats (200-300 g) were anesthetized by intraperitoneal injection of sodium thiopental (100 mg/Kg). Surgical exposure of the appropriate area was begun 5 min. after administration of the anesthetic and samples were removed with syringes containing 50 μl of 0.5 M EDTA to prevent clotting. The blood samples (obtained 15-20 min. after giving the anesthetic) were centrifuged, deproteinized, and analyzed for total glutathione (GSH + GSSG) by the glutathione reductase-DTNB recycling method (9) and for GSH and GSSG by the 2-vinylpyridine modification of this procedure (10).

The blood samples were immediately centrifuged at 10,000 g in a Beckman Microfuge B for 1.5 min. The plasma was rapidly deproteinized by mixing it vigorously with one-half volume of 10% 5-sulfosalicylic acid and centrifuging the mixture at 10,000 g for 2 min. Hemolysis was not detected under conditions in which 0.2% hemolysis could have been found (11). The deproteinized samples were analyzed within 3-5 min. after the samples were withdrawn.

Blood samples were removed from the vein of the left hepatic lobe, from the aorta below the renal arteries, from the renal vein (after a ligature had been placed at the junction of the renal vein with the inferior vena cava), and from the inferior vena cava at a point below the renal veins (after a temporary clamp had been placed above the sample site to prevent mixing with renal venous blood). L-γ-Glutamyl-(o-carboxy)phenylhydrazide (3) was administered subcutaneously (0.5 nmol per kilogram) 20 min. before the blood samples were taken. AT-125 [αS, 5S]-α-amino-3-chloro-4,5-dihydro-5-isoxazoleacetic acid), obtained through the courtesy of Dr. L.J. Hanka of Upjohn, was given by subcutaneous injection (2.5 mmol per kilogram) 2.3 and 5.3 hours prior to taking the blood samples. γ-Glutamyl transpeptidase activity was determined (12) on homogenates of the kidneys obtained immediately after the blood samples were taken.

Results: Total glutathione levels in arterial plasma were 14.5 μM GSH equivalents (Table I), or about 60% of the levels found in arterial plasma from unanesthetized rats (1,2). About 80% of the total was in the form of GSH in arterial plasma of both anesthetized (present study) and unanesthetized rats (1,2). Hepatic vein plasma has a higher level (26 μM) than does arterial plasma; 80% of the total is in the form of GSH. Plasma from inferior vena cava has somewhat less glutathione than does arterial plasma and ∿70% of the total is in the form of GSH. The level of glutathione in renal vein plasma is much lower than

TABLE I

Plasma Glutathione Levels in Different Blood Vessels of Thiopental-Anaesthetized Rats*

Source of Blood Plasma (# of Samples)	Total μM (GSH equiv.)	GSSG μM (GSH equiv.)	GSSG % of Total	GSH μM (GSH equiv.)	GSH % of Total
Hepatic Vein (5)	26.0 ± 4.2	5.0 ± 1.8	19	21.0 ± 4.6	81
Aorta (28)	14.5 ± 2.7	2.7 ± 1.0	19	11.8 ± 2.9	81
Renal Vein (14)	2.6 ± 0.6	0.5 ± 0.4	19	2.1 ± 0.7	81
Inferior Vena Cava (7)	8.1 ± 2.0	2.6 ± 0.9	32	5.5 ± 2.2	68

*The blood samples were drawn and the determinations were performed as described in the text.

TABLE II

Effect of Administration of γ-Glutamyl Transpeptidase Inhibitors on Arterial and Renal Venous Plasma Levels of Glutathione*

Treatment (# of Samples)	Arterial [GSH + GSSG] GSH equiv. μM	Renal Venous [GSH + GSSG] GSH equiv. μM	Disappearance of Arterial Plasma Glutathione
None (Control) (28)	14.5 ± 2.7	2.6 ± 0.6	82%
γ-Glu-(o-COOH)-phenylhydrazide (3)	20.4 ± 3.1	8.8 ± 0.7	57%
AT-125 (2.3 hours) (1)	60.0	32.4	46%
AT-125 (5.3 hours) (2)	80.9 ± 7.3†	49.2 ± 5.1††	39%

*L-γ-glu-(o-COOH)-phenylhydrazide (0.5 mmol/kg) was given subcutaneously. Anesthesia was given 5 min. later. AT-125 (2.5 mmol/kg) was given subcutaneously. Sodium thiopental was given either 2 or 5 hours later (as indicated). The rats given a 5 hour treatment of AT-125 exhibited a pronounced glutathionuria (6.75 mM) and showed little change in the kidney glutathione level; γ-glutamyl transpeptidase inhibition in these rats was greater than 90%, but was not complete.

†54.5 μM GSSG (≈70% of Total); ††45.9 μM GSSG (≈90% of Total).

that found in arterial plasma. These findings reflect substantial translocation of glutathione (as GSH) from the liver to the plasma. The extent of removal of plasma glutathione by the kidney (~80%) is far in excess of the fraction (25-30%) of plasma that undergoes glomerular filtration.

Table II gives glutathione levels in arterial and renal venous plasma in control rats and rats that were injected with γ-glutamyl transpeptidase inhibitors. These inhibitors are known to increase plasma levels of glutathione and to produce glutathionuria (3,6,13); the urinary glutathione arises from glutathione that is translocated out of renal cells into the tubular fluid, and also from plasma glutathione that is filtered through the glomerulus. We found that the percentage of arterial plasma glutathione removed by the kidney decreased substantially after 2 different transpeptidase inhibitors were given. After injection of L-γ-glutamyl-(o-carboxy)phenylhydrazide (a competitive inhibitor), the disappearance of arterial plasma glutathione decreased to 57%. After giving the potent irreversible inhibitor AT-125, the disappearance of arterial plasma glutathione decreased to 39%, a value not far from that expected if glomerular filtration were the only mechanism for removal of plasma glutathione. About 70% of the total glutathione in the arterial plasma of rats treated with AT-125 is in the form of GSSG; the glutathione in renal vein plasma is about 90% GSSG.

Discussion: The finding of high GSH levels in hepatic vein plasma offers direct evidence that liver translocates GSH to the plasma. The plasma glutathione levels found here are much higher than those reported by others (7-9). As discussed (1,2), GSH disappears rapidly from plasma; it is converted to GSSG and other products apparently including mixed disulfides. It is thus important to perform analyses within a few minutes after obtaining the blood samples. It seems probable, in view of the lower total glutathione levels and higher percentages of GSSG

found by others (7-9), that considerable oxidation and reaction of GSH occurred prior to these determinations.

That the level of glutathione in renal plasma is only ~20% of that of arterial plasma is consistent with the conclusion that the kidney has a mechanism, in addition to filtration, for removal of plasma glutathione. Our conclusions are thus in agreement, in this respect, with that of Sies et al (7,8), but our data are quite different. The data (Table II) show that the disappearance of arterial plasma glutathione on passage through the kidney decreases substantially when 2 different types of transpeptidase inhibitors are given. The percentage of glutathione that disappeared from the arterial plasma when the animals were given AT-125 approaches the range of values anticipated if filtration alone were responsible for removal of plasma glutathione. The findings are consistent with the view that the non-filtration mechanism that operates normally probably involves γ-glutamyl transpeptidase not located within the renal tubular lumen. [One cannot unequivocally exlude, however, that the 2 different inhibitors used here fortuitously inhibit another (as yet unknown) mechanism for glutathione removal]. Although many studies have emphasized the high level of γ-glutamyl transpeptidase in the brush-borders of the renal tubular lumen, it is known that transpeptidase occurs elsewhere in the kidney (14,15). Even a low level of activity would suffice to catalyze utilization of arterial plasma glutathione.

Administration of transpeptidase inhibitors increases plasma levels of total glutathione (3,6,13), a large fraction of which is GSSG (Table II). Probably the accumulation of GSH which accompanies inhibition of transpeptidase is followed by increased oxidation to GSSG. Although conceivably GSSG is an intermediate in the major pathway of extracellular GSH metabolism (16), it is more likely that the accumulated GSH undergoes nonenzymatic oxidation to GSSG. Thus, the residual

transpeptidase would be sufficient to produce enough cysteinylglycine (which readily oxidizes to cysteinyl-bis-glycine) to catalyze substantial conversion of GSH to GSSG (17). That the glutathione present in the renal vein plasma of animals treated with AT-125 is ~90% GSSG (compared to ~70% GSSG in arterial plasma) may also be explained by residual transpeptidase. It is relevant that transpeptidase acts more rapidly on GSH than on GSSG in both hydrolytic and transpeptidation reactions (12,18,19).

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