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GLUTATHIONE-DEGRADING CAPACITIES OF LIVER AND KIDNEY IN DIFFERENT SPECIES

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Abstract—Although the liver is recognized as a major site of glutathione (GSH) synthesis, it is thought to play only a minor role in GSH catabolism. This is primarily because in the rat, the most commonly used experimental animal, hepatic γ-glutamyltransferase (γ-GT) activity is very low, whereas kidney activity is quite high. γ-GT is the only enzyme known to catalyze the initial step in GSH degradation. The present work compares γ-GT and dipeptidase activities in liver, kidney, and gallbladder of six mammalian species to assess the importance of hepatobiliary catabolism of GSH, relative to renal degradation. Marked species differences were observed in γ-GT activities, and in kidney to liver (K/L) ratios for both γ-GT concentration (milliunits/mg protein) and whole organ activities (total activity per liver or two kidneys). The K/L concentration ratios for γ-GT activities ranged from 875 in the rat to 15 in the guinea pig. Whole organ γ-GT ratios were ~150 in mouse and rat, and only 2–5 in guinea pig, pig, and macaque. Human K/L ratios calculated from γ-GT activities reported previously were similar to those of the guinea pig. Species differences were also observed in K/L ratios for dipeptidase activities, though these differences were not as large as those for γ-GT. γ-GT and dipeptidase activities were also measured in gallbladders of all species examined (except rat which does not have this organ), and were found to be comparable to those of liver. These results suggest that in species such as the guinea pig and perhaps humans, the liver and biliary tree play a prominent role in GSH turnover. Because of the low hepatic and high renal γ-GT activities of the rat, and because it does not have a gallbladder, this species may not be the best model for studying the catabolism of GSH and GSH conjugates. Use of the rat model may underestimate the contribution of liver, and overestimate that of kidney, in these degradative processes.

It is generally accepted that glutathione (γ-glutamylcysteinylglycine; GSH†) metabolism involves an interorgan cycle: synthesis primarily by the liver, transport via the circulatory system, and degradation predominantly in the kidney [1, 2]. The liver is considered the most important site of GSH synthesis, while the kidney is thought to be the primary site of GSH catabolism because of the high concentration of γ-glutamyltransferase (EC 2.3.2.2) found in this organ [3]. This enzyme, which is also known as γ-glutamyltranspeptidase or γ-GT, is the only enzyme known which can cleave the γ-glutamyl bond, and thus initiate glutathione degradation. Hydrolytic cleavage by γ-GT results in the production of free glutamate and the dipeptide cysteinylglycine; the latter is a substrate for a variety of dipeptidases. The amino acids liberated from GSH can be transported back to the liver for synthesis of new GSH, thus completing the interorgan cycle.

Although the liver is recognized as a major site of GSH synthesis, it has received little attention as a potential site of GSH degradation. This is primarily because most studies of GSH metabolism have been performed using species such as the rat or mouse, in which liver γ-GT activity is very low relative to that of kidney [4]. Recent studies have shown, however, that a large fraction of the GSH synthesized by rat liver is released into bile, and that some of the GSH is metabolized as it travels within the biliary tree [5–7]. In species such as the guinea pig, rabbit and dog, nearly all of the GSH secreted into bile is degraded within the biliary tree, suggesting that extensive metabolism of GSH occurs within the liver [7].

In addition to GSH itself, glutathione conjugates, formed in the liver by the enzyme-catalyzed conjugation of GSH with reactive electrophilic species, are secreted preferentially into bile [8]. It is possible that γ-GT activity within the biliary tree and the gallbladder, an organ which functions to store and concentrate bile, could also attribute to the biliary degradation of GSH and GSH-conjugates. However, the relative contributions of these biliary degradative pathways have not been established.

The present work examines the relative importance of hepatobiliary catabolism of GSH and GSH-conjugates, by comparing γ-GT and dipeptidase activities in liver, gallbladder, and kidney in six mammalian species.

MATERIALS AND METHODS

γ-Glutamyl-*p*-nitroanilide, alanine-*p*-nitroanilide, bovine serum albumin, and glycylglycine were obtained from the Sigma Chemical Co. (St. Louis, MO). γ-GT and dipeptidase activities were measured in homogenates prepared from the livers, kidneys, and gallbladders (where present) of the following

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† Abbreviations: GSH, reduced glutathione; GSSG, glutathione disulfide; γ-GT, γ-glutamyltransferase; and K/L, kidney to liver ratio.

Table 1. Species and tissue differences in γ-GT specific activities

Species	γ-GT Specific activity* (milliunits/mg protein)			K/L ratio
	Kidney	Liver	Gallbladder	
Rat	3325 ± 325 (4)	3.8 ± 0.7 (4)	ND†	875
Mouse	619 ± 45 (4)	1.5 ± 0.2 (5)	2.4 ± 1.3 (3)	413
Rabbit	482 ± 89 (5)	4.8 ± 0.9 (8)	8.4 ± 1.1 (8)	100
Guinea pig	294 ± 23 (8)	19.8 ± 2.3 (13)	5.8 ± 1.3 (5)	15
Pig	123 ± 22 (5)	6.5 ± 1.1 (5)	3.8 ± 0.4 (5)	19
Macaque	454 (2)	9.7 (2)	10.9 (2)	47

* Data are expressed as means ± SE, except for the macaque where the mean of two values is given. Numbers in parentheses = N. One unit = enzyme activity which liberates 1 μmol *p*-nitroaniline from γ-glutamyl-*p*-nitroanilide under assay conditions (see text).

† ND = not determined (rats do not have gallbladders).

species: male Sprague–Dawley rats (200–250 g, Charles River), male BALB/c mice (~30 g, Environmental Health Sciences Center Inbred Mouse Unit, University of Rochester), male New Zealand White rabbits (3.0–3.5 kg, Charles River), male Hartley guinea pigs (350–480 g, Charles River), and female *Macaque fasicularis* (3.5–4.5 kg, University of Rochester Behavioral Toxicology Laboratory). Porcine tissues were obtained from a local slaughterhouse (Conti Packing Co., Henrietta, NY). Animals were anesthetized (mice, 0.1 mg/g sodium pentobarbital; rabbits, ether; guinea pigs, ether or 40 mg/kg ketamine and 5 mg/kg xylazine; macaques, ketamine and sodium pentobarbital) and then killed by exsanguination. Rats were killed by decapitation.

Tissues were excised and rinsed in ice-cold 0.9% NaCl. Gallbladders were cut open and rinsed to remove bile. Liver samples were taken from the left median lobe when possible. Porcine and macaque samples were from liver tissue adjacent to the gallbladder. Kidney samples were either whole organ (mouse, rat, and guinea pig) or sections containing cortex, medulla and papilla. Whole gallbladders were used for all species except pig and macaque. Samples from these species were taken from the gallbladder mid-body region. Mouse gallbladders were pooled for homogenization. All visible liver tissue was removed from gallbladders prior to homogenization. Tissues were weighed, then minced with surgical scissors, and homogenized on ice in 4 vol. of ice-cold 0.9% NaCl (some gallbladders were prepared as 1:10 or 1:15 homogenates) using either Dounce Type A or Polytron (Brinkmann) homogenizers.

Enzyme assays were performed using whole homogenates (gallbladder samples) or homogenates diluted with 0.9% NaCl (1:4 or 1:5 for liver and 1:50 or 1:100 for kidney samples). Protein was determined by the Lowry method using bovine serum albumin as a standard. γ-GT activity was assayed kinetically at 37° with a Gilford 260 spectrophotometer (410 nm) by the method of Orlowski and Meister [9]. γ-Glutamyl-*p*-nitroanilide was used as substrate. A non-kinetic method was used to detect γ-GT in samples with low activity (mouse and rat livers). In this method, whole homogenate and substrate were added to a reaction mixture containing Tris-glycylglycine buffer (total volume = 1.15 mL) and incubated at 37° for 20–45 min. The enzymatic reaction was stopped by precipitating proteins with 100 μL of 12 M acetic acid. After centrifugation in an Eppendorf Microfuge for 2 min, the absorbance of the supernatant fraction was measured at 410 nm and corrected for tissue and substrate blanks. Enzyme activity was calculated using a molar extinction coefficient of $9.9\,M^{-1}\cdot cm^{-1}$. Dipeptidase activity was measured at 410 nm and 37° using the kinetic method of Rankin *et al.* [10] with alanine-*p*-nitroanilide as substrate. Samples with low dipeptidase activity were assayed with a non-kinetic method similar to that described above for γ-GT, with differences only in substrate and reaction mixture (potassium phosphate-$MnCl_2$ buffer was used rather than Tris-glycylglycine).

Whole organ weights were used when possible to determine total organ enzyme levels. When these data were not available, organ weights were estimated from body weights [mouse: liver weight (LW) = 5.3% body weight (BW), kidney weight (KW) = 1.6% BW; rabbit: LW = 3.3% BW, KW = 0.5% BW; guinea pig: LW = 3.5% BW, KW = 0.8% BW; macaque: LW = 2.5% BW, KW = 0.5% BW]. Porcine liver weights were estimated to be six times those of total kidney weight.

RESULTS

Table 1 lists γ-GT specific activities (milliunits/mg protein) for kidney, liver, and gallbladder in six different species. One unit is defined as that enzyme activity which will liberate 1 μmol of *p*-nitroaniline/min under assay conditions. The concentration of γ-GT in rat kidney was relatively high (Table 1), as previously observed [11–13]. In contrast, the activity of γ-GT in rat liver was very low, approximately 0.1% of that in the kidney (Table 1). γ-GT specific activities were higher in kidney than in liver for all species examined, although these differences were not as marked as in the rat (Table 1). For example, the guinea pig, which had the highest liver γ-GT specific activity, had a kidney to liver (K/L) concentration ratio of only 15 (Table 1). This lower K/L ratio in the guinea pig was due to both a lower renal γ-GT specific activity (294 vs 3325 milliunits/mg protein) and a higher hepatic γ-GT activity (19.8 vs 3.8 milliunits/mg protein) as compared to the rat.

Table 2. Species differences in whole organ γ-GT activities

Species	Whole organ γ-GT activity* (units/organ)			K/L ratio
	Kidney	Liver	Gallbladder	
Rat	1010 ± 41 (4)	7.1 ± 1.4 (4)	ND†	142
Mouse	60.0 ± 4.2 (4)	0.47 ± 0.05 (5)	0.001 ± 0.001 (3)	128
Rabbit	1119 ± 186 (5)	71.0 ± 9.1 (8)	0.43 ± 0.10 (8)	16
Guinea pig	148 ± 13 (9)	46.5 ± 4.2 (13)	0.13 ± 0.04 (5)	3
Pig	3800 ± 769 (5)	1600 ± 255 (5)	6.0 ± 0.7 (5)	2
Macaque	988 (2)	181 (2)	1.7 (2)	5

* Data are expressed as means ± SE, except for the macaque where the mean of two values is given. Numbers in parentheses = N. One unit = enzyme activity which liberates 1 μmol *p*-nitroaniline from γ-glutamyl-*p*-nitroanilide under assay conditions (see text).

† ND = not determined (rats do not have gallbladders).

In fact, of the six species examined, the rat had the highest kidney specific activity (5- to 27-fold higher than the other species) and one of the lowest hepatic specific activities (Table 1).

Although human tissues were not analyzed in the present study, Shaw *et al.* [14] reported human γ-GT specific activities to be 590 and 27 milliunits/mg protein for kidney cortex and liver respectively. These values can be used to estimate a K/L concentration ratio of approximately 22 for human γ-GT. This is similar to K/L values calculated for guinea pig and pig (Table 1).

Gallbladder γ-GT specific activities were comparable to those of liver for all five species (rats, which do not have gallbladders, are excluded here). No human gallbladder γ-GT data are available for comparison.

Whole organ γ-GT activities were calculated for kidney, liver, and gallbladder (Table 2), to determine the relative capacities of these organs to degrade glutathione. Rat and mouse kidneys had much higher γ-GT activity than livers from these species, with K/L ratios of approximately 150. This ratio was much smaller in the other species. For example, pig and guinea pig whole kidney γ-GT activities were only 2- to 3-fold higher than liver. Whole gallbladder activities were low relative to the other two organs (Table 2).

Whole organ γ-GT can be estimated for human kidney and liver from the activities reported by Shaw *et al.* [14], and assuming organ weights of 350 g for two kidneys and 1750 g for liver (based on an average body weight of 70 kg). These values give γ-GT activities of 41,300 and 10,500 units for kidney and liver, respectively, and a K/L ratio of 3.9, which is similar to the whole organ ratios for pig and guinea pig. The actual ratio in humans is probably lower than this since the kidney γ-GT activity reported by Shaw and coworkers was for kidney cortex, where γ-GT activity is concentrated, rather than for whole kidney.

Dipeptidase activities (milliunits/mg protein) for kidney, liver, and gallbladder are listed in Table 3. As for γ-GT, dipeptidase specific activities were higher in kidney, as compared to liver or gallbladder tissue. This difference was greatest in the rabbit (with a K/L ratio of 69) and smallest in the mouse (Table 3). Gallbladder dipeptidase specific activities were higher than those in liver in all species examined except pig.

Whole organ dipeptidase activities were calculated (Table 4) to compare the relative degradative capacities of liver and kidney. In general, whole organ activities were higher in kidney than liver, although these differences were not as large as those observed for γ-GT (Table 2). In fact, the dipeptidase whole organ K/L ratio for pig was only slightly larger than 1. Whole gallbladder activities were low when compared to either kidney or liver.

DISCUSSION

This work illustrates marked species differences in the distribution of γ-GT activity between liver and kidney, suggesting species-specific interorgan patterns of GSH metabolism. In addition, these findings indicate that the liver may be a site of extensive degradation of GSH and GSH-conjugates in species such as the guinea pig and possibly humans, which have low K/L ratios for γ-GT activity. Efflux of GSH into plasma and bile is responsible for almost all of hepatic GSH turnover. Thus, species which have higher hepatic γ-GT activities may have a significant portion of released GSH degraded within the liver before degradation can occur in the kidney or small intestine [7].

Among the species examined, the rat is quite unusual in its pattern of γ-GT distribution—high renal activity with relatively low hepatic activity. Although γ-GT activities were higher in kidney than liver for all species examined, the magnitude of these interorgan differences was most striking in the rat, which had a K/L γ-GT concentration ratio of about 900. Even when comparing whole organ activities, this ratio is still approximately 150 for the rat, and only 2–5 for the pig, guinea pig and macaque, a non-human primate. Estimates of whole organ K/L ratios from human liver and kidney γ-GT activities reported previously [14], indicate that the relative distribution of this enzyme in humans resembles that of guinea pig or macaque rather than that of rat or mouse. Therefore, the rat and mouse may not be the best species to use as models of the catabolism of GSH and GSH-conjugates.

In addition to γ-GT activity, rates of transport of the substrates across hepatocyte canalicular or

Table 3. Species and tissue differences in dipeptidase specific activities

Species	Dipeptidase specific activity* (milliunits/mg protein)			K/L ratio
	Kidney	Liver	Gallbladder	
Rat	65.2 ± 4.7 (4)	3.3 ± 0.3 (4)	ND†	20
Mouse	31.4 ± 3.1 (4)	5.4 ± 0.7 (5)	9.4 ± 0.1 (3)	6
Rabbit	48 ± 7 (5)	0.70 ± 0.04 (5)	2.1 ± 0.2 (4)	69
Guinea pig	155 ± 20 (8)	5.5 ± 0.5 (13)	14.3 ± 0.2 (5)	28
Pig	78 ± 5 (5)	8.3 ± 1.2 (5)	3.6 ± 0.4 (5)	9
Macaque	60 (2)	3.7 (2)	4.1 (2)	16

* Data are expressed as mean ± SE, except for the macaque where the mean of two values is given. Numbers in parentheses = N. One unit = enzyme activity which liberates 1 μmol *p*-nitroaniline from alanine-*p*-nitroanilide under assay conditions (see text).
† ND = not determined (rats do not have gallbladders).

Table 4. Species differences in whole organ dipeptidase activities

Species	Whole organ dipeptidase activity* (units/organ)			K/L ratio
	Kidney	Liver	Gallbladder	
Rat	20.2 ± 1.1 (4)	6.1 ± 0.4 (4)	ND†	3.3
Mouse	3.0 ± 0.3 (4)	1.7 ± 0.2 (5)	0.005 ± 0.001 (3)	1.8
Rabbit	112 ± 17 (5)	12.6 ± 1.0 (5)	0.07 ± 0.01 (4)	8.9
Guinea pig	77 ± 10 (9)	13.2 ± 1.5 (13)	0.33 ± 0.06 (5)	5.8
Pig	2428 ± 203 (5)	2178 ± 490 (5)	5.6 ± 0.7 (5)	1.1
Macaque	136 (2)	71 (2)	0.61 (2)	1.9

* Data are expressed as means ± SE, except for the macaque where the mean of two values is given. Numbers in parentheses = N. One unit = enzyme activity which liberates 1 μmol *p*-nitroaniline from alanine-*p*-nitroanilide under assay conditions (see text).
† ND = not determined (rats do not have gallbladders).

basolateral membranes, and localization of the enzyme within the tissue are important determinants of the extent and site of hepatic catabolism of GSH and GSH-conjugates in different species. By inhibiting γ-GT activity within the biliary tree, it has been shown that this is an important site for GSH degradation [5–7]. Stein and coworkers [15] found that GSH was the main thiol in mouse, hamster and rat bile, whereas cysteinylglycine was predominant in bile from rabbit and guinea pig. They correlated the larger percentage of cysteinylglycine in the bile of the latter two species with the higher activity of γ-GT in their livers.

Since GSH-conjugates are excreted preferentially into bile [8], biliary metabolism of these compounds will occur to a greater extent in species with γ-GT activity concentrated on canalicular and bile ductular membranes. In studies on the biliary excretion of methylmercury, which is secreted as a GSH complex [16], the GSH–methylmercury complex was found to be the major metabolite in species with low hepatic γ-GT activity, such as the rat [15, 17]. However, in species with high hepatic γ-GT levels, methylmercury was excreted primarily as the cysteinylglycine-*S*-complex [15, 17]. Gregus and coworkers [18] report similar findings in a study of the biliary excretion of GSH conjugates of acetaminophen. These findings indicate that GSH-conjugates can also be metabolized by γ-GT activity within the biliary tree. In addition to GSH-conjugates, GSSG is also secreted preferentially into bile [2], along with a large amount of reduced GSH [5], all of which are potential substrates for γ-GT and dipeptidase activity present in biliary and gallbladder tissues.

Histochemical techniques have demonstrated species differences in the localization of γ-GT within the liver. For example, γ-GT activity in the normal adult rat is localized to bile duct epithelium and occasional bile canaliculi [19, 20]. Strong canalicular staining for γ-GT has been observed in the rabbit, while the guinea pig shows faint staining along the bile canaliculi and intense sinusoidal staining (unpublished observation). Because of these differences in enzyme distribution within the liver, the rat and the guinea pig probably have distinct patterns of hepatic GSH metabolism. The abundant sinusoidal γ-GT activity in the guinea pig indicates that a significant fraction of the GSH released from hepatocytes into the circulation could be broken down within the guinea pig liver. In contrast, since the rat has no detectable sinusoidal γ-GT activity, most of the GSH secreted into plasma by the liver will be degraded by the kidney, as has been reported [1, 2].

The presence of γ-GT and dipeptidase activities in the gallbladder (Tables 1 and 3) suggests that this organ also plays a role in the degradation of GSH and GSH-conjugates excreted into bile. As bile is

stored and concentrated in the gallbladder, the potential exists for significant metabolism of these compounds. This possibility is currently being investigated in this laboratory.

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REFERENCES

1. McIntyre TM and Curthoys NP, The interorgan metabolism of glutathione. *Int J Biochem* **12**: 543–551, 1980.
2. Akerboom TPM and Sies H, Transport of glutathione, glutathione disulfide, and glutathione conjugates across the hepatocyte plasma membrane. In: *Methods in Enzymology* (Eds. Fleischer S and Fleischer B), Vol. 173, pp. 523–533. Academic Press, San Diego, CA, 1989.
3. Meister A and Tate SS, Glutathione and related γ-glutamyl compounds: Biosynthesis and utilization. *Annu Rev Biochem* **45**: 559–604, 1976.
4. Orlowski M and Wilk S, Metabolism of γ-glutamyl amino acids and peptides in mouse liver and kidney *in vivo*. *Eur J Biochem* **71**: 549–555, 1976.
5. Ballatori N, Truong A, Ma A and Boyer JL, Determinants of glutathione efflux and biliary GSH/GSSG ratio in perfused rat liver. *Am J Physiol* **256**: G482–G490, 1989.
6. Ballatori N, Jacob R and Boyer JL, Intrabiliary glutathione hydrolysis: A source of glutamate in bile. *J Biol Chem* **261**: 7860–7865, 1986.
7. Ballatori N, Jacob R, Barrett C and Boyer JL, Biliary catabolism of glutathione and differential reabsorption of its amino acid constituents. *Am J Physiol* **254**: G1–G7, 1988.
8. Wahllander A and Sies H, Glutathione *S*-conjugate formation from 1-chloro-2,4-dinitrobenzene and biliary *S*-conjugate excretion in the perfused rat liver. *Eur J Biochem* **96**: 441–446, 1979.
9. Orlowski M and Meister A, γ-Glutamyl-*p*-nitroanilide: A new convenient substrate for determination and study of L- and D-γ-glutamyltranspeptidase activities. *Biochim Biophys Acta* **73**: 676–679, 1963.
10. Rankin BB, McIntyre TM and Curthoys NP, Brush border membrane hydrolysis of *S*-benzyl-cysteine-*p*-nitroanilide, an activity of amino-peptidase M. *Biochem Biophys Res Commun* **96**: 991–996, 1980.
11. Tate SS, Enzymes of mercapturic acid formation. In: *Enzymatic Basis of Detoxication* (Ed. Jakoby W), Vol. II, pp. 95–120. Academic Press, New York, 1980.
12. Igarashi T, Satoh T, Ueno K and Kitagawa H, Changes of gamma-glutamyltranspeptidase activity in the rat during development and comparison of the fetal liver, placental and adult liver enzymes. *Life Sci* **29**: 483–491, 1981.
13. Taniguchi M and Inoue M, Ontogenic changes in metabolism and transport of glutathione in the rat. *J Biochem (Tokyo)* **100**: 1457–1463, 1986.
14. Shaw LM, London JW and Petersen LE, Isolation of γ-glutamyltransferase from human liver, and comparison with the enzyme from human kidney. *Clin Chem* **24**: 905–915, 1978.
15. Stein AF, Gregus Z and Klaassen CD, Species variations in biliary excretion of glutathione-related thiols and methylmercury. *Toxicol Appl Pharmacol* **93**: 351–359, 1988.
16. Ballatori N and Clarkson TW, Biliary transport of glutathione and methylmercury. *Am J Physiol* **244**: G435–G441, 1983.
17. Urano T, Naganuma A and Imura N, Species differences in biliary excretion of methylmercury—Role of non-protein sulfhydryls in bile. *Res Commun Chem Pathol Pharmacol* **62**: 339–351, 1988.
18. Gregus Z, Cherukury M and Klaassen CD, Species variation in toxication and detoxication of acetaminophen *in vivo*: A comparative study of biliary and urinary excretion of acetaminophen metabolites. *J Pharmacol Exp Ther* **244**: 91–99, 1988.
19. Albert Z, Orlowski M and Szewczuk A, Histochemical demonstration of gamma-glutamyl transpeptidase. *Nature* **191**: 767–768, 1961.
20. Rutenburg AM, Kim H, Fischbein JW, Hanker JS, Wasserkrug HL and Seligman AM, Histochemical and ultrastructural demonstration of γ-glutamyl transpeptidase activity. *J Histochem Cytochem* **17**: 517–526, 1969.