Biochemical Pharmacology, Vol. 33, No. 11, pp. 1801–1807, 1984.
Printed in Great Britain.

0006-2952/84 $3.00 + 0.00

DIFFERENTIAL DISTRIBUTION OF GLUTATHIONE AND GLUTATHIONE-RELATED ENZYMES IN RABBIT KIDNEY

POSSIBLE IMPLICATIONS IN ANALGESIC NEPHROPATHY*

JANARDANAN MOHANDAS,† JOCELYN J. MARSHALL, GEOFFREY G. DUGGIN, JOHN S. HORVATH and DAVID J. TILLER
Renal Unit, Royal Prince Alfred Hospital, Camperdown 2050, Sydney, N.S.W., Australia

(*Received* 6 *August* 1983; *accepted* 3 *November* 1983)

Abstract—Whole tissue reduced glutathione (GSH) concentration was found to be lowest in rabbit renal inner medulla and progressively higher in outer medulla and cortex. Activities of cytosolic glutathione reductase in inner medulla and outer medulla were similar, and each was only approximately 50% of that of cortex. Whole tissue and microsomal γ-glutamyl transpeptidase activities were high in cortex and outer medulla but were low in inner medulla. Cytosolic activity of selenium-dependent glutathione peroxidase (GPx-I) was similar in both outer medulla and inner medulla but was only 50% of that of cortex. Activity of cytosolic selenium-independent glutathione peroxidase (GPx-II) was highest in cortex and lowest in inner medulla (~15% of cortex and ~50% of outer medulla). Cytosolic glutathione *S*-transferase activity with 1-chloro-2,4-dinitrobenzene as substrate was high in all three regions of kidney. With 1,2-dichloro-4-nitrobenzene and 1,2-epoxy-(4-nitrophenoxy)propane as substrates, cytosolic glutathione *S*-transferase activities were very low in cortex, outer medulla, and inner medulla. Microsomal activities of glutathione reductase, GPx-I, GPx-II and glutathione *S*-transferases were much lower than activities of corresponding cytosolic enzymes. Activities of the glutathione peroxidases in renal inner medulla would hence be expected to cause little interference to prostaglandin endoperoxide synthetase mediated cooxidative activation of paracetamol. It has been demonstrated that the paracetamol metabolite can react rapidly with GSH, forming not only glutathione conjugate but also paracetamol itself and oxidized glutathione. Low GSH concentrations, as well as low activities of glutathione reductase, GPx-I, GPx-II, and γ-glutamyl transpeptidase, may therefore render the inner medullary region of kidney particularly vulnerable to paracetamol-related analgesic nephropathy.

Long-term intake of either paracetamol or phenacetin, in combination with other antipyretic analgesics, has been implicated in the development of the kidney disease commonly referred to as analgesic nephropathy [1–3]. Renal papillary necrosis (RPN) has been recognized as the initial lesion caused by analgesics [4]. Phenacetin is converted rapidly into its major metabolite, paracetamol, after ingestion [5]. Prolonged intake of phenacetin and paracetamol in small doses has failed to induce RPN in experimental animals [1, 2, 6], but the situation in humans may well be different. Renal papillary tissue damage is produced in rabbits with phenacetin [7] and in rats with a mixture of phenacetin and other analgesics [8] after long-term treatment with large doses. However, paracetamol is generally regarded as the compound which is primarily responsible for causing RPN as observed in analgesic nephropathy [1, 2]. It is possible that the mechanisms in the development of paracetamol-related analgesic nephropathy in man could result from a multiplicity of factors. The disease of analgesic nephropathy could be initiated by prolonged high concentrations of paracetamol in renal inner medulla, resulting from the intake of its parent compound phenacetin, when combined with frequent episodes of physiological hydropenia [2]. We have demonstrated [9, 10] that metabolic activation of paracetamol occurs predominantly in renal inner medulla in comparison to outer medulla and cortex through the mediation of the prostaglandin hydroperoxidase activity of the enzyme, prostaglandin endoperoxide synthetase (PGES, EC 1.14.99.1). Other investigators have also observed the metabolic activation of paracetamol by microsomal PGES in rabbit kidney medulla [11, 12] and ram seminal vesicular PGES-mediated formation of the glutathione conjugate of paracetamol [13]. PGES in its highly purified state has both fatty acid cyclooxygenase (arachidonic acid → prostaglandin G_2) and prostaglandin hydroperoxidase (prostaglandin G_2 → prostaglandin H_2) activities [14, 15]. Investigations on the effect of selenium-dependent glutathione peroxidase (GPx-I, EC 1.11.1.9) on PGES have demonstrated that GPx-I can inhibit PGES in the presence of GSH [16, 17] and that this inhibition is dependent upon the relative activities of the two enzymes [18]. It was proposed, on the basis of these observations, that the inhibitory effect of GPx-I on PGES became effective when prostaglandin G_2 (PGG_2) was converted rapidly into prostaglandin H_2 (PGH_2) by GPx-I to a concentration lower than the minimum concentration required for the continual activation of the cyclooxygenase activity of PGES

* Part of this work was presented at the Twelfth International Congress of Biochemistry, Perth, Australia, August 15–21, 1982.

† Author to whom reprint requests should be sent.

[16, 17]. Selenium-independent glutathione peroxidase (GPx-II) activity [19] arises from certain isozymes of glutathione *S*-transferases (EC 2.5.1.18), and its effect on PGES has, so far, not been investigated. However, both GPx-I and GPx-II have been demonstrated to participate in the removal of organic hydroperoxides *in vivo*, and to cause oxidized glutathione (GSSG) release [20].

In rabbit renal inner medulla, where prostaglandin biosynthetic activity is very high [21], GPx-I and/or GPx-II might play a regulatory role by participating in the conversion of PGG_2 to PGH_2, and this, in turn, could then be an important factor in the metabolic activation of different xenobiotics, including paracetamol. Reduction of intracellular GSSG is dependent upon glutathione reductase (EEC 1.6.4.2) activity [22] and, when the capacity of the latter is exceeded, efflux of GSSG occurs from the cell [23]. High efficiency of the kidney in the recovery of extracellular GSSG is well established [24–26], and this is primarily due to high level activity of renal γ-glutamyl transpeptidase (EC 2.3.2.2) which initiates the breakdown of GSSG [24–26]. These aspects prompted us to investigate the differential distribution of GSH concentrations and activities of glutathione-related enzymes in rabbit kidney to examine the possible mechanisms for the origin and development of paracetamol-related analgesic nephropathy.

MATERIALS AND METHODS

Chemicals. GSH, GSSG, 5,5′-dithiobis(2-nitrobenzoic acid), glutathione reductase (type III), reduced nicotinamide adenine dinucleotide phosphate (NADPH), bovine serum albumin (Fraction V), glycylglycine and DL-dithiothreitol were purchased from the Sigma Chemical Co. (St. Louis, MO). Cumene hydroperoxide was obtained from ICN Pharmaceuticals Inc. (Plainview, NY). 1-Chloro-2,4-dinitrobenzene (CDNB) was obtained from BDH Chemicals Ltd. (Poole, U.K.). 1,2-Dichloro-4-nitrobenzene (DCNB) was obtained from the Aldrich Chemical Co. (Milwaukee, WI). L-γ-Glutamyl-*p*-nitroanilide and 1,2-epoxy-(4-nitrophenoxy)propane (ENP) were supplied by the Calbiochem-Behring Corp. (La Jolla, CA). Metaphosphoric acid and deoxycholic acid were purchased from Hopkin & Williams (Chadwell Heath, Essex, U.K.). Other chemicals were of the highest purity available from commercial sources.

Animals. Male New Zealand white rabbits (4 months old) were purchased from Tillside Rabbit Stud (Bargo, N.S.W., Australia) and were allowed to acclimatize for at least 1 week before use. Rabbits which had continual access to food were killed by a single blow on the neck, and kidney GSH measurements were carried out as described below. Twenty-four-hour-starved animals were used for the preparation of renal subcellular fractions required for enzyme activity determinations. These animals were killed by decapitation after they had lost consciousness in a carbon dioxide chamber.

Preparation of subcellular fractions. Immediately following death of the rabbits (four in each group), the kidneys were removed and placed in an ice-cold solution of 0.9% NaCl. All subsequent operations were carried out at 0–4°. Kidneys were separated into cortex, outer medulla and inner medulla, as described previously [27]. The tissues were then weighed, minced finely, and homogenized for 15 sec with a polytron homogenizer in 3 vol. of potassium phosphate buffer (0.1 M, pH 7.8) containing dithiothreitol (0.1 mM) and glycerol (20%). For the γ-glutamyl transpeptidase assay alone, this buffer was replaced with Tris–HCl (0.01 M, pH 7.5). The homogenates were centrifuged at 9000 *g* for 15 min, and the supernatant fractions were filtered through glass wool before centrifuging at 105,000 *g* for 1 hr. The supernatants (cytosol fractions) were again filtered through glass wool and were stored in small aliquots at −80°. The pellets (microsomal fractions) were rinsed twice with 1.15% KCl, covered with the same solution, and stored at −80°. Just before use, these pellets were resuspended by homogenization with a Potter–Elvehjem Teflon–glass homogenizer in 1.15% KCl and recentrifuged at 105,000 *g* for 1 hr. These washed microsomes were suspended in either potassium phosphate buffer (0.1 M, pH 7.8) or Tris–HCl (0.05 M, pH 8.0), the latter for the γ-glutamyl transpeptidase assay.

Measurement of GSH. This was carried out by a modification of the method of Ellman [28]. Immediately following exsanguination, kidneys were removed from each rabbit and separated into cortex, outer medulla and inner medulla; small portions of each region (~250 mg) were placed into liquid nitrogen. While still frozen, these tissues were weighed and then homogenized in a mixture of potassium phosphate (0.1 M) containing EDTA (5 mM), adjusted to pH 8.0 and 25% metaphosphoric acid (3.75:1, v/v), using a Potter–Elvehjem Teflon–glass homogenizer. This homogenate was centrifuged at 10,000 *g* for 20 min, and sulfhydryl concentrations were measured by the addition of 5,5′-dithiobis(2-nitrobenzoic acid) into appropriate aliquots of the supernatant fractions. Results were expressed as μmoles GSH per g tissue wet weight.

Glutathione reductase. Activity was measured by a modification of the method of Carlberg and Mannervik [29]. The assay mixture (1 ml) contained potassium phosphate buffer (0.1 M, pH 7.6), NADPH (0.1 mM), EDTA (0.5 mM), GSSG (1 mM) and 100–200 μl of cytosol or microsomal suspension (6 mg protein/ml). The reaction was carried out at 30° and was initiated by the addition of enzyme source. Enzyme activity was determined by measuring the disappearance of NADPH at 340 nm and was expressed as nmoles NADPH oxidized per min per mg protein.

γ-Glutamyl transpeptidase. Activity was determined by the method described by Tate and Meister [30] with modification as follows. For whole tissue assays, tissues were homogenized in 5 vol. of Tris–HCl (0.01 M, pH 7.5). Whole tissue homogenates, cytosols and microsomes were then preincubated with deoxycholic acid (1%) for 15 min at 25°. The assay system (1.0 ml) consisted of glycylglycine (20 mM), L-γ-glutamyl-*p*-nitroanilide (2.5 mM), NaCl (75 mM), enzyme source (10–200 μl) and Tris–HCl buffer (0.05 M, pH 8.0). The reaction was initiated by the addition of L-γ-glutamyl-*p*-nitroanilide. The reaction was followed spectrophotometrically at

Table 1. GSH concentrations and glutathione reductase activities in different regions of rabbit kidney*

	GSH (μmoles GSH/g tissue wet weight) (N = 4)	Glutathione reductase (nmoles NADPH oxidized/min/mg protein)	
		Cytosol (N = 8)	Microsomes (N = 7)
Cortex	9.2 ± 0.7	191 ± 6	23.6 ± 2.8
Outer medulla	5.8 ± 0.4†	112 ± 5†	12.0 ± 2.5†
Inner medulla	4.4 ± 0.5†‡	95 ± 5†	19.1 ± 2.5‡

* Results are expressed as means ± S.E. The number of determinations is in parentheses.
† Significantly different from cortex ($P < 0.05$).
‡ Significantly different from outer medulla ($P < 0.05$).

37° from the increase in absorption at 410 nm. The enzyme activity was expressed as nmoles of product formed per min per mg protein.

Glutathione peroxidases (GPx-I and GPx-II). Activities were determined by a modification of the method described by Lawrence and Burk [19]. GPx-I utilizes hydrogen peroxide and organic hydroperoxides, such as cumene hydroperoxide, as substrates. GPx-II can act on organic hydroperoxides but not on hydrogen peroxide. This difference in their abilities to utilize hydrogen peroxide was employed for the determination of the activities of GPx-I and GPx-II [19]. The assay medium contained potassium phosphate buffer (0.05 M, pH 7.0), EDTA (1 mM), NaN_3 (1 mM), glutathione reductase (1 E.U.), GSH (1 mM), NADPH (0.2 mM), 10–100 μl of enzyme source (6 mg protein/ml) and either hydrogen peroxide (0.25 mM) or cumene hydroperoxide (1.5 mM). The enzyme activity was determined at 37° by measuring the dissappearance of NADPH at 340 nm and was expressed as nmoles NADPH oxidized per min per mg protein. The activity with hydrogen peroxide as substrate represented GPx-I, and the difference between cumene hydroperoxide and hydrogen peroxide initiated activities represented GPx-II [19].

Glutathione S-transferases. Activities were determined by adaptations of the method described by Habig *et al.* [31]. For the assay with CDNB as the substrate, the reaction mixture (1 ml) consisted of potassium phosphate buffer (0.1 M, pH 6.5), GSH (1 mM), CDNB (1 mM) and 10–100 μl of enzyme source (6 mg protein/ml). A similar method was employed for the measurement of glutathione *S*-transferase activity with DCNB, and the assay medium contained potassium phosphate buffer (0.1 M, pH 7.5), GSH (5 mM), DCNB (1 mM) and 100–300 μl of enzyme source (6 mg protein/ml). Using ENP as substrate, the glutathione *S*-transferase assay was carried out in a reaction mixture containing potassium phosphate buffer (0.1 M, pH 6.5), GSH (5 mM), 300–500 μl of enzyme source (6 mg protein/ml) and ENP (0.5 mM). These assays were carried out at 37° and were followed with a recording spectrophotometer. The enzyme activity in each case was expressed as nmoles product formed per min per mg protein. Protein concentrations were measured by the method of Lowry *et al.* [32] with bovine serum albumin (Fraction V) as standard. Results (means ± S.E.) expressed in Tables 1–4 were corrected for blank values and represented four to eleven different determinations. The data were analyzed using Student's *t*-test.

RESULTS

Tissue concentrations of GSH and activities of glutathione reductase. Acid-soluble sulfhydryl concentrations determined by the method of Ellman represent mostly GSH. More than 95% of total tissue glutathione in kidney was demonstrated to be GSH [33]. Among the three regions of kidney, inner medulla had the lowest GSH content which represented less than 50% of cortex (Table 1). All regions

Table 2. γ-Glutamyl transpeptidase activities in different regions of rabbit kidney*

	γ-Glutamyl transpeptidase (nmoles product formed/min/mg protein)		
	Whole tissue (N = 7)	Microsomes (N = 4)	Cytosol (N = 4)
Cortex	411 ± 74	1417 ± 300	10.3 ± 2.9
Outer medulla	199 ± 29†	981 ± 215	6.0 ± 1.4
Inner medulla	26 ± 5†‡	31 ± 5†‡	0†‡

* Results are expressed as means ± S.E. The number of determinations is in parentheses.
† Significantly different from cortex ($P < 0.05$).
‡ Significantly different from outer medulla ($P < 0.05$).

Table 3. Activities of selenium-dependent and selenium-independent glutathione peroxidases (GPx-I and GPx-II respectively) in different regions of rabbit kidney*

	Glutathione peroxidases (nmoles NADPH oxidized/min/mg protein)			
	GPx-I		GPx-II	
	Cytosol (N = 9)	Microsomes (N = 4)	Cytosol (N = 9)	Microsomes (N = 4)
Cortex	404 ± 19	24 ± 4	442 ± 28	20.8 ± 8.3
Outer medulla	197 ± 16†	21 ± 3	158 ± 16†	9.8 ± 4.6
Inner medulla	192 ± 9†	24 ± 5	72 ± 8†‡	5.0 ± 4.7

* Results are expressed as means ± S.E. The number of determinations is in parentheses.
† Significantly different from cortex ($P < 0.05$).
‡ Significantly different from outer medulla ($P < 0.05$).

had relatively high cytosolic glutathione reductase activity (Table 1). In terms of differential distribution, the cortical region had twice the glutathione reductase activity of the medullary region. Glutathione reductase activity was several-fold higher in cytosol than in microsomes from every region of kidney (Table 1). Microsomal glutathione reductase activity was much lower in outer medulla than in cortex and inner medulla.

γ-Glutamyl transpeptidase activities. Activities of γ-glutamyl transpeptidase were found to be high in both whole tissue and microsomal samples from cortex and outer medulla and low in inner medullary samples of whole tissue and microsomes (Table 2), as observed previously by Shimada *et al.* [34].

Glutathione peroxidases—GPx-I and GPx-II. As seen in Table 3, the activity of cytosolic GPx-I in cortex was higher than that in outer medulla or in inner medulla (50% of cortex). The microsomal GPx-I activity was found to be comparatively low in all three regions. The activity of cytosolic GPx-II fell markedly from cortex to inner medulla (1/6 of cortex) and that in the outer medulla occupied an intermediate position (Table 3). In microsomal fractions, the activity of GPx-II was found to be very low, but showed an apparent quantitative differentiation among the three regions (Table 3), although not statistically significant ($P > 0.05$).

Glutathione S-transferases. Activities of glutathione *S*-transferases with three substrates, CDNB (cytosol and microsomes), DCNB (cytosol) and ENP (cytosol), in cortex, outer medulla and inner medulla of rabbit kidney, as presented in Table 4, demonstrated that both qualitative and quantitative differentiation occurred generally in the three regions. With certain exceptions, the activities of glutathione *S*-transferases were much higher in cortex than in inner medulla. Cytosolic glutathione *S*-transferase activities with CDNB as substrate were relatively high in all three regions, although the corresponding enzyme activity was higher in cortex than that in either outer medulla or inner medulla (3/5 of cortex). The microsomal glutathione *S*-transferase activities with CDNB as substrate were low in all three regions and again this enzyme activity in cortex was much higher than that present in either outer medulla or inner medulla. Cytosolic glutathione *S*-transferase activities with DCNB as substrate were low in all three regions, but they were significantly higher in the inner medulla than in the outer medulla. Activities of glutathione *S*-transferase in cytosols with ENP as substrate were also low in cortex, outer medulla and inner medulla, and this enzyme activity in cortex represented twice that present in either inner or outer medulla (Table 4). Microsomal glutathione *S*-transferase activities towards DCNB and

Table 4. Activities of glutathione *S*-transferases in different regions of rabbit kidney*

	Glutathione *S*-transferases (nmoles glutathione conjugate formed/min/mg protein)			
	Substrate			
	CDNB		DCNB	ENP
	Cytosol (N = 9)	Microsomes (N = 4)	Cytosol (N = 9)	Cytosol (N = 11)
Cortex	2470 ± 171	72 ± 10	7.0 ± 0.5	17.4 ± 1.4
Outer medulla	1274 ± 117†	24 ± 2†	6.0 ± 0.8	8.8 ± 0.6†
Inner medulla	1467 ± 94†	20 ± 2†	9.4 ± 1-2‡	9.9 ± 1.0†

* Results are expressed as means ± S.E. The number of determinations is in parentheses.
† Significantly differently from cortex ($P < 0.05$).
‡ Signficantly different from outer medulla ($P < 0.05$).

ENP were either absent or were not detectable by the methods employed here for their measurement (data not shown).

DISCUSSION

A schematic view of the activation of paracetamol in renal inner medulla and of the importance of GSH status and GSH-related enzymes in the prevention of paracetamol toxicity is presented in Fig. 1. The two major enzymes involved in metabolic activation of xenobiotics in kidney are cytochrome P-450 mixed-function oxidase (Cyt. P-450 MFO) and PGES, and their patterns of differential distribution are opposite to one another within the kidney [9, 27, 35]. While Cyt. P-450 MFO occurs maximally

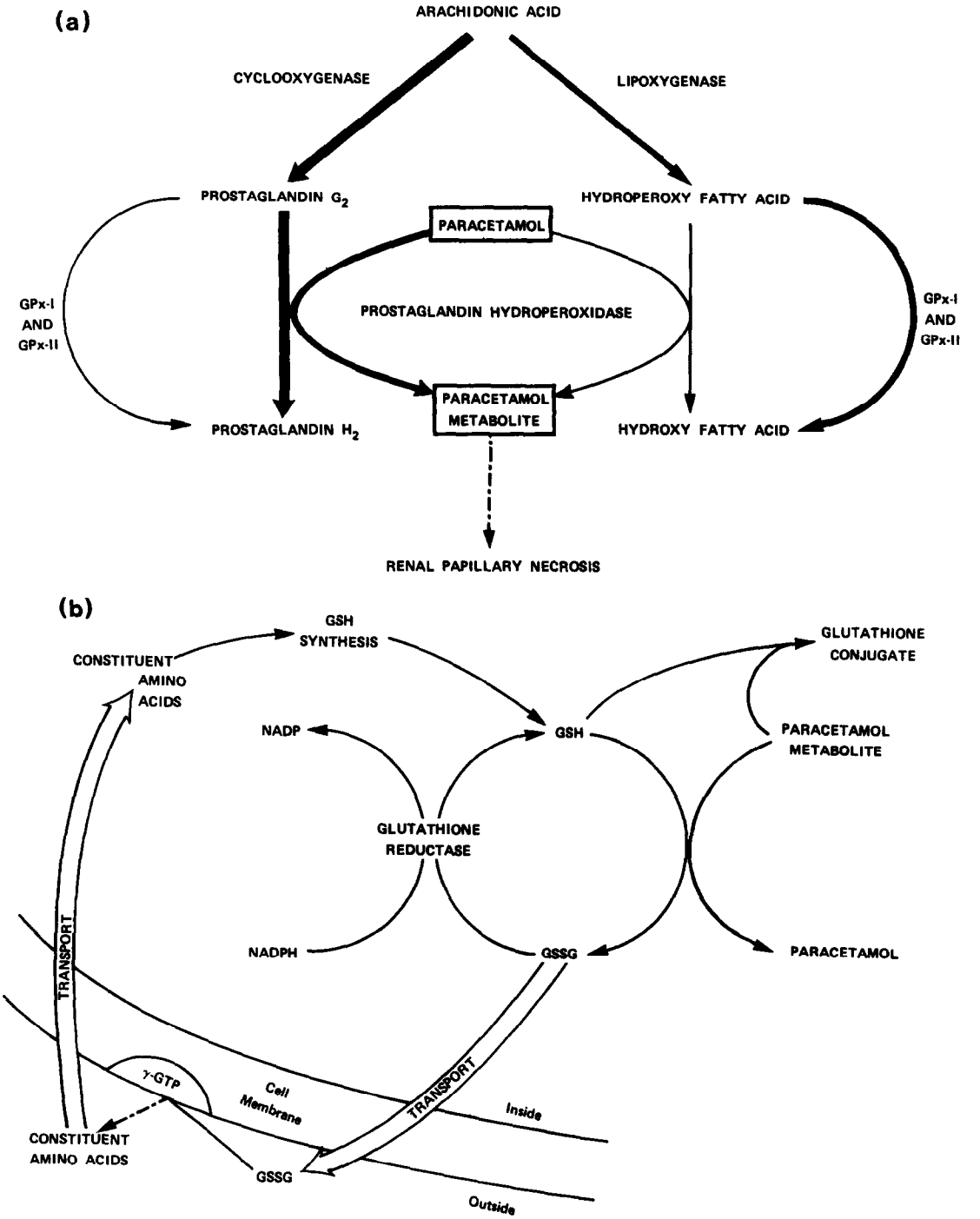

Fig. 1. Schematic representation of the mechanisms of activation and detoxification of paracetamol in renal inner medulla. (a) Possible interaction between glutathione peroxidases (GPx-I and GPx-II) and prostaglandin hydroperoxidase during activation of paracetamol. (b) Importance of GSH status in the detoxification of paracetamol.

in cortex, it is not detectable in inner medulla. On the other hand, PGES occurs predominantly in inner medulla and its activity is very low in cortex. In previous studies, paracetamol was demonstrated to be activated differentially by both these enzymes in rabbit kidney, while functioning as whole enzyme systems [9] and as hydroperoxidases [10]. In renal inner medulla, the induction of paracetamol-related analgesic nephropathy appears to be initiated by PGES-mediated activation of paracetamol [9, 10].

Due to the ability of GPx-I to convert PGG_2 into PGH_2, it was suggested previously [36] that glutathione peroxidase could be the enzyme responsible for the *in vivo* conversion of PGG_2 into PGH_2 during prostaglandin biosynthesis. The activities of GPx-I and GPx-II in inner medulla (Table 3) did not appear to affect activation of paracetamol in this region since it was observed that incubation of renal inner medullary slices of rabbit with radiolabeled paracetamol led to very high covalent binding to protein [37]. The sharing of the conversion of PGG_2 into PGH_2 by both glutathione peroxidase and prostaglandin hydroperoxidase would influence the extent of cooxidative activation of xenobiotics, including paracetamol, since glutathione peroxidase activity has not been known to mediate the oxidative conversion of any xenobiotic. Hence, only that proportion of PGG_2 which would be converted into PGH_2 by prostaglandin hydroperoxidase would be available to participate in the cooxidation of xenobitotics. Whilst prostaglandin hydroperoxidase can function in the absence of GSH, GPx-I and GPx-II can only function in the presence of GSH [19]. A fall in GSH would impair the participation of glutathione peroxidase and therefore would make the participation of prostaglandin hydroperoxidase more dominant in the conversion of PGG_2 into PGH_2 and the concurrent metabolic activation of xenobiotics including paracetamol. The paracetamol metabolite formed by the PGES-mediated activation has been found to react rapidly with GSH *in vitro* [9] and hence could cause severe depletion of intracellular GSH by similar activation *in vivo* in inner medulla which had the lowest GSH status within the kidney (Table 1). Resultant impairment of glutathione peroxidase activity would then increase the PGES-mediated activation of paracetamol and release more reactive metabolite.

Arachidonic-acid-utilizing lipoxygenase was reported recently to be present in the renal medulla of rabbit [38]. Lipoxygenases have been strongly implicated in producing hydroperoxy fatty acids as initial products from polyunsaturated fatty acids including arachidonic acid [39]. These hydroperoxy fatty acids have been known to undergo rapid conversion into hydroxy fatty acids through the mediation of glutathione peroxidases (GPx-I or GPx-II) and GSH. Impairment of this reduction system due to a fall in glutathione peroxidase activity, or in GSH status, would cause enhancement of the concentration of hydroperoxy fatty acids [39–41]. During the formation of large amounts of reactive metabolites of paracetamol in inner medulla, the resultant rapid depletion of GSH would prevent efficient conversion of hydroperoxy fatty acids, formed by medullary lipoxygenase, to hydroxy fatty acids by glutathione peroxidases, and these hydroperoxy fatty acids might become additional substrates for prostaglandin hydroperoxidase-mediated cooxidation of paracetamol (Fig. 1a).

GSH concentration is very critical for the prevention of paracetamol toxicity [42, 43]. Intake of paracetamol invariably results in depletion of GSH, and tissue necrosis occurs only when the concentration of GSH falls below the safety threshold [43]. The role of GSH in protecting the cell by directly reacting with the paracetamol metabolite and forming a glutathione conjugate has long been recognized [42, 43]. Recent studies by Moldeus *et al.* [12] indicated that most of the paracetamol metabolite reacted directly with GSH forming the parent compound itself and GSSG. Thus, although the reaction between GSH and the paracetamol metabolite results in immediate depletion of GSH, the GSH converted to GSSG could be recovered, unlike the GSH utilized to form the glutathione conjugate of paracetamol [44]. GSSG normally is reduced to GSH intracellularly by glutathione reductase, requiring NADPH [22]. During the high level of activation of paracetamol which can occur in renal inner medulla, this availability of NADPH necessary for the regeneration of GSH from GSSG could be affected by the reaction which has been found to occur between NADPH and the paracetamol metabolite [45]. This reaction between NADPH and the paracetamol metabolite, producing paracetamol, was reported to be more pronounced in the absence of GSH [45]. When the capacity of the reduction process of GSSG to GSH is exceeded either by low activity or glutathione reductase, or due to lack of availability of NADPH, the excess GSSG would move into the extracellular compartment [23]. There is only one enzyme, γ-glutamyl transpeptidase, which can initiate the breakdown of GSSG by cleaving the γ-glutamyl moiety [46]. Following this, cysteinylglycine dipeptidase would complete the conversion of GSSG into component amino acids [24–26]. The sites of high activity of both these enzymes are known to be on the extracellular side of the brush border membrane in the kidney [24–26]. The amino acid components of GSSG would then be transported into the intracellular compartment for synthesis to GSH [24–26] and thus replenish part of the lost GSH. A definite relationship was demonstrated recently between the activity of γ-glutamyl transpeptidase in liver tissue and its ability to recover GSSG from the extracellular compartment [47]. The diminished GSH status and the high PGES activity in inner medulla suggested that, during PGES-mediated activation of paracetamol, the GSH fall, as well as the concurrent rise in GSSG occuring in inner medulla, would be more pronounced than in outer medulla or in cortex. The GSSG transported into the extracellular compartment would only be minimally acted upon by γ-glutamyl transpeptidase in inner medulla compared to cortex and outer medulla, which have high activities of this enzyme. Hence, most of the GSSG moving into the extracellular compartment of inner medulla would be lost (Fig. 1b).

It is not known whether or not glutathione *S*-transferases would play a direct role in the prevention of the toxicity of the reactive metabolite of paracet-

amol. With regard to many other reactive metabolites, glutathione *S*-transferases would act to prevent toxicity and carcinogenicity [48, 49]. Data from Tables 3 and 4 indicated that the proportion of cytosolic GPx-II activity arising from cytosolic glutathione *S*-transferase activity (with CDNB as the substrate) in inner medulla was the lowest among the three regions of kidney. The pharmacological significance of this is not very clear at present. The biphasic effect of paracetamol on rabbit renal inner medullary prostaglandin biosynthesis [50] could play an important role in the magnitude of PGES-mediated activation of paracetamol, since regional concentrations of paracetamol were found to reach highest concentrations in the inner medulla within the kidneys of dogs after the ingestion of phenacetin or paracetamol, indicating the presence of a renal concentration gradient for paracetamol [51, 52].

The data presented here, along with our interpretations of that data on the basis of previous observations in the area of analgesic-induced damage in the inner medulla, provide a possible mechanism for the genesis of paracetamol-related analgesic nephropathy.

Acknowledgements—The authors are grateful to Mrs. Sonia Richmond for assistance in the preparation of this manuscript. This work was supported by a grant from the National Health and Medical Research Council of Australia.

REFERENCES

1. G. G. Duggin, *Kidney Int.* **18**, 553 (1980).
2. T. Murray and M. Goldberg, *A. Rev. Med.* **26**, 537 (1975).
3. J. H. Stewart, *Kidney Int.* **13**, 72 (1978).
4. M. H. Gault, T. C. Rudwal, W. D. Engles and J. B. Dosseter, *Ann. intern. Med.* **68**, 906 (1968).
5. B. Brodie and J. Axelrod, *J. Pharmac. exp. Ther.* **97**, 58 (1949).
6. E. A. Molland, *Kidney Int.* **13**, 5 (1978).
7. E. Clausen, *Lancet* **ii**, 123 (1964).
8. C. Abrahams, A. H. Rubenstein and N. W. Levin, *Nature, Lond.* **200**, 695 (1963).
9. J. Mohandas, G. G. Duggin, J. S. Horvath and D. J. Tiller, *Toxic. appl. Pharmac.* **61**, 252 (1981).
10. J. Mohandas, G. G. Duggin, J. S. Horvath and D. J. Tiller, *Res. Commun. Chem. Path. Pharmac.* **34**, 69 (1981).
11. J. A. Boyd and T. E. Eling, *J. Pharmac. exp. Ther.* **219**, 659 (1981).
12. P. Moldeus, B. Andersson, A. Rahimtula and M. Berggren, *Biochem. Pharmac.* **31**, 1363 (1982).
13. P. Moldeus and A. Rahimtula, *Biochem. biophys. Res. Commun.* **96**, 496 (1980).
14. F. J. van der Ouderaa, M. Buytenhek, D. H. Nugteren and D. A. van Dorp, *Biochim. biophys. Acta* **487**, 315 (1977).
15. S. Ohki, N. Ogino, S. Yamamoto and O. Hayaishi, *J. biol. Chem.* **254**, 829 (1979).
16. H. W. Cook and W. E. M. Lands, *Nature, Lond.* **260**, 630 (1976).
17. M. E. Hemler and W. E. M. Lands, *J. biol. Chem.* **255**, 6253 (1980).
18. A. M. Hanel and W. E. M. Lands, *Biochem. Pharmac.* **31**, 3307 (1982).
19. R. A. Lawrence and R. F. Burk, *Biochem. biophys. Res. Commun.* **71**, 952 (1976).
20. R. F. Burk, K. Nishiki, R. A. Lawrence and B. Chance, *J. biol. Chem.* **253**, 43 (1978).
21. C. Larsson and E. Anggard, *Eur. J. Pharmac.* **21**, 30 (1973).
22. E. Beutler, in *Glutathione* (Eds. L. Flohe, H. Ch. Benohr, H. Sies, H. D. Waller and A. Wendel), p. 109. Geng. Thieme, Stuttgart (1974).
23. B. H. Lauterburg, C. V. Smith, H. Hughes and J. R. Mitchell, *Trends pharmac. Sci.* **3**, 245 (1982).
24. R. Hahn, A. Wendel and L. Flohe, *Biochim. biophys. Acta* **539**, 324 (1978).
25. N. P. Curthoys and R. P. Hughey, *Enzyme* **24**, 383 (1979).
26. D. P. Jones, P. Moldeus, A. H. Stead, K. Ormstad, H. Jornvall and S. Orrenius, *J. biol. Chem.* **254**, 2787 (1979).
27. T. V. Zenser, M. B. Mattammal and B. B. Davis, *J. Pharmac. exp. Ther.* **207**, 719 (1978).
28. G. L. Ellman, *Archs Biochem. Biophys.* **82**, 70 (1959).
29. I. Carlberg and B. Mannervik, *J. biol. Chem.* **250**, 5475 (1975).
30. S. Tate and A. Meister, *J. biol. Chem.* **249**, 7593 (1974).
31. W. H. Habig, M. J. Pabst and W. B. Jakoby, *J. biol. Chem.* **249**, 7130 (1974).
32. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. L. Randall, *J. biol. Chem.* **193**, 265 (1951).
33. F. Tietze, *Analyt. Biochem.* **27**, 502 (1969).
34. H. Shimada, H. Endou and F. Sakai, *Jap. J. Pharmac.* **32**, 121 (1982).
35. T. V. Zenser, M. B. Mattammal and B. B. Davis, *J. Pharmac. exp. Ther.* **208**, 418 (1979).
36. D. H. Nugteren and E. Hazelhof, *Biochim. biophys. Acta* **326**, 448 (1973).
37. S. Joshi, T. V. Zenser, M. B. Mattammal, C. A. Herman and B. B. Davis, *J. Lab. clin. Med.* **92**, 924 (1978).
38. T. S. Winokur and A. R. Morrison, *J. biol. Chem.* **256**, 10221 (1981).
39. R. W. Bryant, T. C. Simon and J. M. Bailey, *J. biol. Chem.* **257**, 14937 (1982).
40. R. W. Bryant and J. M. Bailey, *Biochem. biophys. Res. Commun.* **92**, 268 (1980).
41. C. W. Parker, C. M. Fischman and H. J. Wedner, *Proc. natn. Acad. Sci. U.S.A.* **77**, 6870 (1980).
42. W. Z. Potter, S. S. Thorgeirsson, D. J. Jollow and J. R. Mitchell, *Pharmacology* **12**, 129 (1974).
43. J. R. Mitchell, D. J. Jollow, W. Z. Potter, J. R. Gillette and B. B. Brodie, *J. Pharmac. exp. Ther.* **87**, 211 (1973).
44. E. M. Kosower and N. S. Kosower, in *Glutathione: Metabolism and Function* (Eds. I. M. Arias and W. B. Jakoby), p. 139. Raven Press, New York (1976).
45. J. A. Hinson, L. R. Pohl, T. J. Monks and J. R. Gillette, *Life Sci.* **29**, 107 (1981).
46. A. Meister, S. S. Tate and L. L. Ross, in *The Enzymes of Biological Membranes* (Ed. A. Martonosi), Vol. 3, p. 315. Plenum Press, New York (1976).
47. T. Igarashi, T. Satoh, K. Hoshi, K. Ueno and H. Kitagawa, *Life Sci.* **31**, 2655 (1982).
48. W. B. Jakoby, *Adv. Enzymol.* **46**, 383 (1978).
49. L. F. Chasseaud, *Adv. Cancer Res.* **29**, 175 (1979).
50. G. G. Duggin and J. Mohandas, in *Advances in Pharmacology and Therapeutics II* (Eds. H. Yoshida, Y. Hagihara and S. Ebashi), Vol. 5, p. 77. Pergamon Press, Oxford (1982).
51. L. W. Bluemle, Jr. and M. Goldberg, *J. clin. Invest.* **47**, 2507 (1968).
52. G. H. Mudge, M. W. Gemborys and G. G. Duggin, *J. Pharmac. exp. Ther.* **206**, 218 (1978).