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Immunohistochemical Localization of Glutamine Transaminase K, a Rat Kidney Cysteine Conjugate β-Lyase, and the Relationship to the Segment Specificity of Cysteine Conjugate Nephrotoxicity

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SUMMARY

Rat kidney glutamine transaminase K is a major rat kidney cysteine conjugate β-lyase and is a key enzyme in the nephrotoxicity of some cysteine conjugates. However, it has not been demonstrated that the β-lyase is present in the target cells. Furthermore, although all segments of the proximal tubule are affected by high doses of nephrotoxic cysteine conjugates, the S3 segment is the most sensitive. Because heterogeneous distribution of the β-lyase could account for the enhanced sensitivity, antibody raised against rat kidney cysteine conjugate β-lyase has been prepared and used to investigate the distribution of the enzyme in kidney and other tissues. The data show that the enzyme is highest in rat kidney, consistent with enzyme activity data. By immunohistochemical staining, no enzyme is present in the glomeruli or distal tubular elements of the kidney. The enzyme is present only in the target cells, the renal proximal tubular epithelium. However, the distribution of the β-lyase within the proximal tubule is not consistent with the hypothesis that a higher concentration of the enzyme in the S3 segment accounts for the greater sensitivity of S3 to nephrotoxic cysteine conjugates compared to S1 and S2. Several alternative hypotheses are discussed.

Recent evidence has confirmed the role of mercapturate biosynthesis in the activation of xenobiotics to toxic species and these studies are the subject of several recent reviews (1–4). The cysteine conjugate β-lyase pathway has received much attention regarding this issue because mercapturate biosynthesis is a major source of toxic xenobiotic cysteine conjugates. A key step in the nephrotoxicity of cysteine conjugates is their metabolism, via β-cleavage, by the pyridoxal phosphate-dependent enzyme cysteine conjugate β-lyase (1–5). Upon β-cleavage, both DCVC and PCBDC yield reactive fragments that covalently bind to cellular macromolecules (6–9). The binding of the reactive fragment is believed to initiate the toxicity (1–4, 6). Alkylation may be involved in the toxicity of other cysteine conjugates, but further work is necessary to prove this mechanism.

The toxicity of cysteine conjugates that are metabolized by the β-lyase pathway is largely restricted to the proximal tubule of the kidney (9–11), although other organ abnormalities have been noted (10, 11). An intriguing feature of the toxicity in the rat proximal tubule is that the lesion appears to be focussed in the area of the pars recta that contains the proximal straight tubule, or S3 segment. Although at high doses damage to the pars convoluta, which is composed of the S1 and S2 segments, has been observed (11), the data for DCVC and PCBDC clearly suggest that S3 is much more sensitive (9–11). Furthermore, S3 specific toxicity is also induced by DCVC *in vitro* in rat kidney slices, which maintain the tubular architecture (12). This suggests that the sensitivity is an intrinsic property of that segment and cannot be explained by factors such as blood flow.

Tubular damage from other xenobiotics that may form toxic cysteine conjugates is also restricted to S3. *S*-(2-Chloro-1,1,2-trifluoroethyl)-L-cysteine has been proposed to be the causative agent in the nephrotoxicity of chlorotrifluoroethylene gas (13), and inhalation exposure to the gas results in a pattern of focal necrosis in the pars recta (14). Therefore, the pars recta sensitivity may be a common feature for the nephrotoxicity of a number of cysteine conjugates.

As yet, an adequate explanation for the segment specificity of these nephrotoxic conjugates is not available. However, an obvious hypothesis is that localization of the β-lyase in S3 segment of the proximal tubule could account for the increased

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ABBREVIATIONS: DCVC, *S*-(1,2-dichlorovinyl)-L-cysteine; PCBDC, *S*-(1,2,3,4,4-pentachlorobutadienyl)-L-cysteine; OSOM, outer stripe of the outer medulla; ABC, avidin-biotin-peroxidase complex; PBS, phosphate-buffered saline.

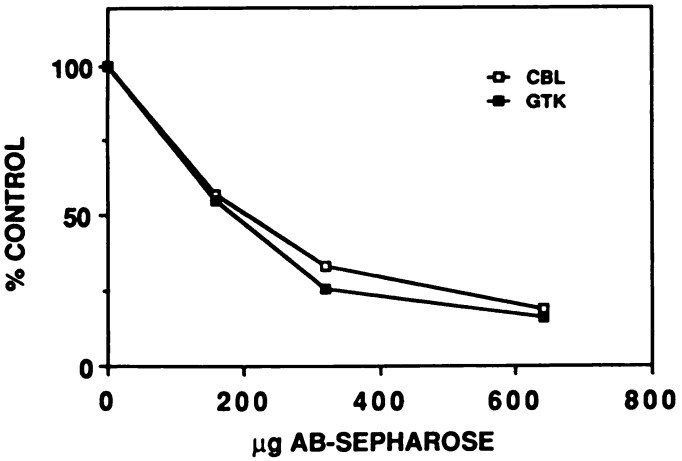

Fig. 1. Immunoadsorption of glutamine transaminase K and cysteine conjugate β-lyase activity from rat kidney cytosol. Aliquots of antibody-Sepharose were added to rat kidney cytosol and immunoadsorbed protein was removed as described in Experimental Procedures. Glutamine transaminase (*GTK*) cysteine conjugate β-lyase (*CBL*) activity and protein content were determined on the remaining cytosol. The percentage of the enzyme activity remaining in the cytosol was calculated as a percentage of the specific activity (nmol/10 min·mg of protein) in the control. Addition of Sepharose to which no antibody was attached had no affect on the activity of either enzyme (data not shown). β-Lyase activity was assayed using [^{14}C]DCVC with 0.5 mM α-keto-δ-methiolbutyrate added to the incubations, according to Stevens *et al.* (5).

sensitivity. In this study, we test this hypothesis using immunohistochemical techniques and antibody raised against glutamine transaminase K, a rat kidney cytosolic cysteine conjugate β-lyase. Purification of both the mitochondrial and cytosolic forms of this enzyme suggest that it is the enzyme responsible for the majority of DCVC metabolism (5, 15). When DCVC is administered *in vivo* (11) or *in vitro* (12) in slice models, the S3 segment is found to be the most sensitive. The antibody recognized both the mitochondrial and cytosolic forms of the β-lyase, both of which have been shown to be isozymes of glutamine transaminase K (5, 15). However, other enzymes with different substrate specificity may also be present (15, 16). The results do not show any major differences in the distribution of glutamine transaminase K in the proximal tubule. Alternative explanations for the segment specificity of cysteine conjugate toxicity are discussed. We have also investigated the tissue distribution of the β-lyase protein and the data are compared with the distribution of the β-lyase and glutamine transaminase activities.

Experimental Procedures

Materials. The Vectastain ABC immunohistochemical staining kit (Vector Laboratories, Burlingame, CA) was used for immunohistochemical studies of tissue section. Rabbit anti-goat IgG horseradish peroxidase-conjugated antibody as well as horseradish peroxidase color development reagent were purchased from Bio-Rad (Richmond, CA). Phenylalanine and α-keto-γ-methiolbutyrate were from Sigma Chemical Co. (St. Louis, MO). The sources of antibody reagent for the immunoblotting and immunohistochemical studies are listed below. All other reagents were of the highest quality available and were used without further purification. Sprague-Dawley rats were used as the animal model.

Antibody and antibody-Sepharose conjugate preparation. Goat anti-rat cysteine conjugate β-lyase was prepared as follows. Purified rat kidney glutamine transaminase K (cysteine conjugate β-lyase; Ref. 5) was subjected to nondenaturing gel electrophoresis as described by Maizel (16). Gels were fixed and stained and slices containing 100 μg of purified protein were mixed with complete Freund's adjuvant and injected subcutaneously in a domestic goat. Two booster injections of gel slices containing 50 μg of purified enzymes were administered in incomplete Freund's adjuvant approximately 3 weeks apart. Plasma was collected by plasmaphoresis and the IgG fraction was prepared by ammonium sulfate precipitation.

After dialysis against PBS, affinity-purified antibody was prepared from the IgG fraction. Purified glutamine transaminase K (0.5 mg) was covalently linked to Activated CH-Sepharose (Pharmacia Fine Chemicals, Upsala, Sweden) according to the manufacturer's instructions. The IgG fraction was applied and the column was washed with 20 mM potassium phosphate containing 0.5 M sodium chloride. Specifically bound antibody was then eluted with 0.2 M citrate/potassium phosphate buffer, pH 2.7. Affinity purified anti-β-lyase antibody gave qualitatively similar results to unpurified antibody, but nonspecific background on immunoblots was less with affinity-purified material.

Antibody was also covalently attached to activated CH-Sepharose and the resulting conjugate was used for immunoadsorption experiments. For this purpose, the IgG fraction was used. The antibody-Sepharose conjugate was dried to a moist cake using a Buchner funnel.

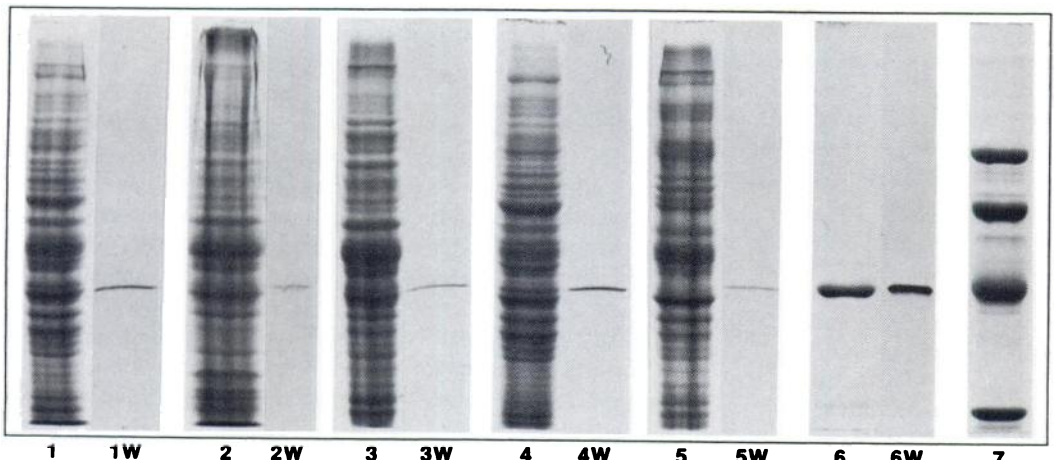

Fig. 2. Immunoblotting and SDS-PAGE analysis of glutamine transaminase K in rat kidney subcellular fractions. Protein (100 μg) from homogenate (*H*), nuclear fraction (*Nu*), mitochondria (*Mt*), cytosol (*C*), and microsomes (*Mc*) were compared with purified glutamine transaminase K (*Pure En.*; 1 μg) by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and immunoblotting with affinity-purified antibody to glutamine transaminase K. The protein separation (*numbered lanes*) is shown adjacent to an identical lane that was probed by immunoblotting (*W*, Western blot). Protein standards were from the top of the gel, phosphorylase B (94,000), bovine serum albumin (67,000), ovalbumin (43,000), and carbonic anhydrase (30,000).

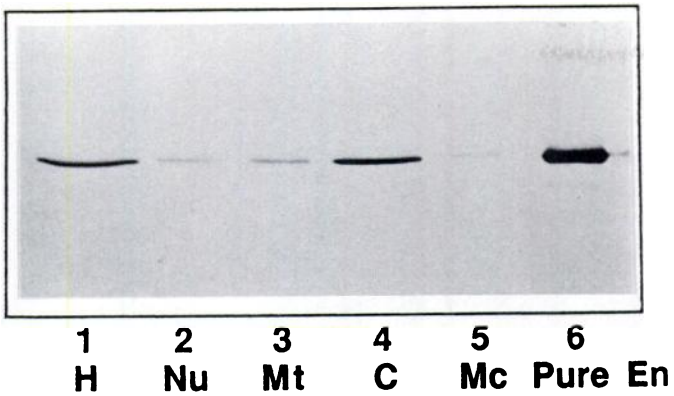

Fig. 3. Subcellular distribution of glutamine transaminase K by blotting. An amount of protein equivalent to the percentage of total homogenate protein (100 μg) contributed by that fraction was loaded in separate lanes; nuclear (*Nu*), 12 μg; mitochondria (*Mt*), 18 μg; cytosol (*C*), 64 μg; and microsomes (*Mc*), 6 μg. Purified enzyme (1 μg) is shown in *lane 6*.

Aliquots were added to rat kidney cytosol, prepared as described (5), and the suspension was vortexed. The suspension was incubated at 0° for 1 hr, with periodic mixing, and then centrifuged to separate the antibody-Sepharose from the cytosol. Samples of the supernatant from the centrifugation step were taken for protein and enzyme activity analysis. Unmodified Sepharose served as the control.

Immunohistochemical staining. In initial studies, we compared tissues fixed with formalin, Bouin's fixative, or ethanol. Although the segmental distribution of the staining was similar with all the fixatives, ethanol proved to be superior in preserving antigenicity. Tissues used for immunohistochemical staining were fixed in ethanol for 24 hr processed routinely, and embedded in paraffin. Serial 5 μM sections were cut and stained with hematoxylin and eosin or used for immunohistochemical studies. Staining for β-lyase was performed using the ABC method described by Hsu *et al.* (17) as outlined in the Vectastain ABC kit (Vector Laboratories). The sections were first cleared of paraffin and placed in absolute methanol containing 1.5% H_2O_2 for 20 min to ablate endogenous peroxidase activity. Tissue sections were rinsed in 0.1 M PBS (pH 7.2) for 20 min and incubated for 20 min with diluted normal goat serum. The sections were then treated for 60 min at room temperature with a 1:50 dilution of the affinity-purified goat anti-rat β-lyase antibody described above. This dilution of antibody was chosen after testing several dilutions. Although the staining was reduced at higher dilutions of antibody, the relative staining in the various segments of the tubule did not change. The tissue sections were subsequently washed in 10 mM Tris·HCl (pH 7.6) for 10 min and treated with a biotinylated anti-goat antibody. After a 10 min wash in Tris·HCl (pH 7.6), the sections were incubated with the Avidin-horseradish peroxidase complex. The sections were again washed in Tris·HCl (pH 7.6) and peroxidase localization was achieved by treatment with 0.05% diaminobenzidine tetrahydrochloride prepared in 0.1 M Tris·HCl (pH 7.2) to which 0.03% H_2O_2 had been added. The sections were counterstained with Gill's hematoxylin, differentiated using acid-alcohol. The specificity of the staining was examined using appropriate controls. Negative controls included the use of primary antibody preadsorbed with the immunizing antigen in place of the primary antibody. In addition, a background control, in which PBS was substituted for the primary antibody, was run for all sections. After the demonstration of renal staining for β-lyase, a section of kidney was included with all nonrenal tissue to serve as a positive control.

Enzyme assays. Cysteine conjugate β-lyase activity was assayed with [^{14}C]DCVC as the substrate. [^{14}C]DCVC was synthesized by the method of Hayden *et al.* (18). Metabolism was determined using the extraction assay previously reported (5, 6). All assays were done in the presence of 0.5 mM α-keto-δ-methiolbutyrate unless specified otherwise. Glutamine transaminase K activity was measured according to Cooper (19). In both cases the activities were determined at 37°.

Immunoblotting. For immunoblot analysis, proteins were separated on a 10% sodium dodecyl sulfate gels as described by Maizel (16). Protein was transferred to nitrocellulose paper using a Bio-Rad Mini-blot apparatus (Bio-Rad). After transfer, the nitrocellulose paper was washed in Tris-buffered saline according to the manufacturer's instructions and blocked for 30 min with 5% powdered milk dissolved in the same buffer. Affinity-purified goat anti-rat cysteine conjugate β-lyase antibody was added to the nitrocellulose paper at a dilution of 1:400 and incubated overnight. After washing twice with Tris-buffered saline, affinity-purified rabbit anti-goat IgG-horseradish peroxidase conjugate was diluted 1:300 and incubated with the nitrocellulose paper for 1 hr. Immunoreactive protein was visualized using the peroxidase staining procedure and horseradish peroxidase color development reagent as described by the manufacturer (Bio-Rad).

Results

We investigated the specificity of the antibody used in the immunohistochemical studies by two methods. The first procedure employed the immunoadsorption of both cysteine conjugate β-lyase activity and glutamine transaminase K from rat kidney cytosol, using antibody that had been linked covalently to Sepharose beads (Fig. 1). Cytosol was chosen as a source of enzyme because the majority of β-lyase acitivity with DCVC is present in that fraction from rat kidney (16). As noted in the introduction, the antibody also reacts with the mitochondrial form of the enzyme (15). Increasing amounts of antibody-Sepharose were added to rat kidney cytosol and then removed by centrifugation. The individual data points in Fig. 1 represent the amounts of cysteine conjugate β-lyase and glutamine transaminase K activity that remained in the supernatant after immunoprecipitation with antibody-Sepharose. The two activities are removed in a coordinate fashion, suggesting that the antibody recognized one protein having both activities, as suggested by Stevens *et al.* (5).

The specificity of the antibody was also determined by immunoblotting protein from rat kidney cortex homogenates and subcellular fractions with antibody to rat kidney cysteine conjugate β-lyase (glutamine transaminase K). In order to obtain high titer specific antibody, the IgG fraction from the goat serum was first affinity purified, using a column of Sepharose 4B to which purified rat kidney cytosolic cysteine conjugate β-lyase had been covalently bound (see Experimental Procedures). The affinity purification improved the antibody titer against purified cysteine conjugate β-lyase from 1:100 to 1:800, as determined by dot-blots on nitrocellulose paper (data not shown). The affinity-purified antibody was used to probe nitrocellulose paper to which 100 μg of protein from each fraction, separated by gel electrophoresis in sodium dodecyl sulfate, had been electrophoretically transferred. The data in Fig. 2 show that the antibody recognized only one protein band in the whole homogenate and that the molecular weight of the antigen band in the crude homogenate was similar to that of the purified enzyme. The staining was most intense in the cytosolic fraction, but significant staining was also seen in the mitochondrial and microsomal fractions (Fig. 2). The nuclear fraction showed faint but recognizable reactivity. For the immunoblot shown in Fig. 3, each lane of the gel was loaded with an amount of protein equivalent to the percentage of total homogenate protein contained in a particular subcellular fraction. Thus, the intensity of the staining reflects the subcellular distribution of the protein. Cytosol contained most of the immunoreactive protein. This is consistent with the cytosolic distribution of β-lyase

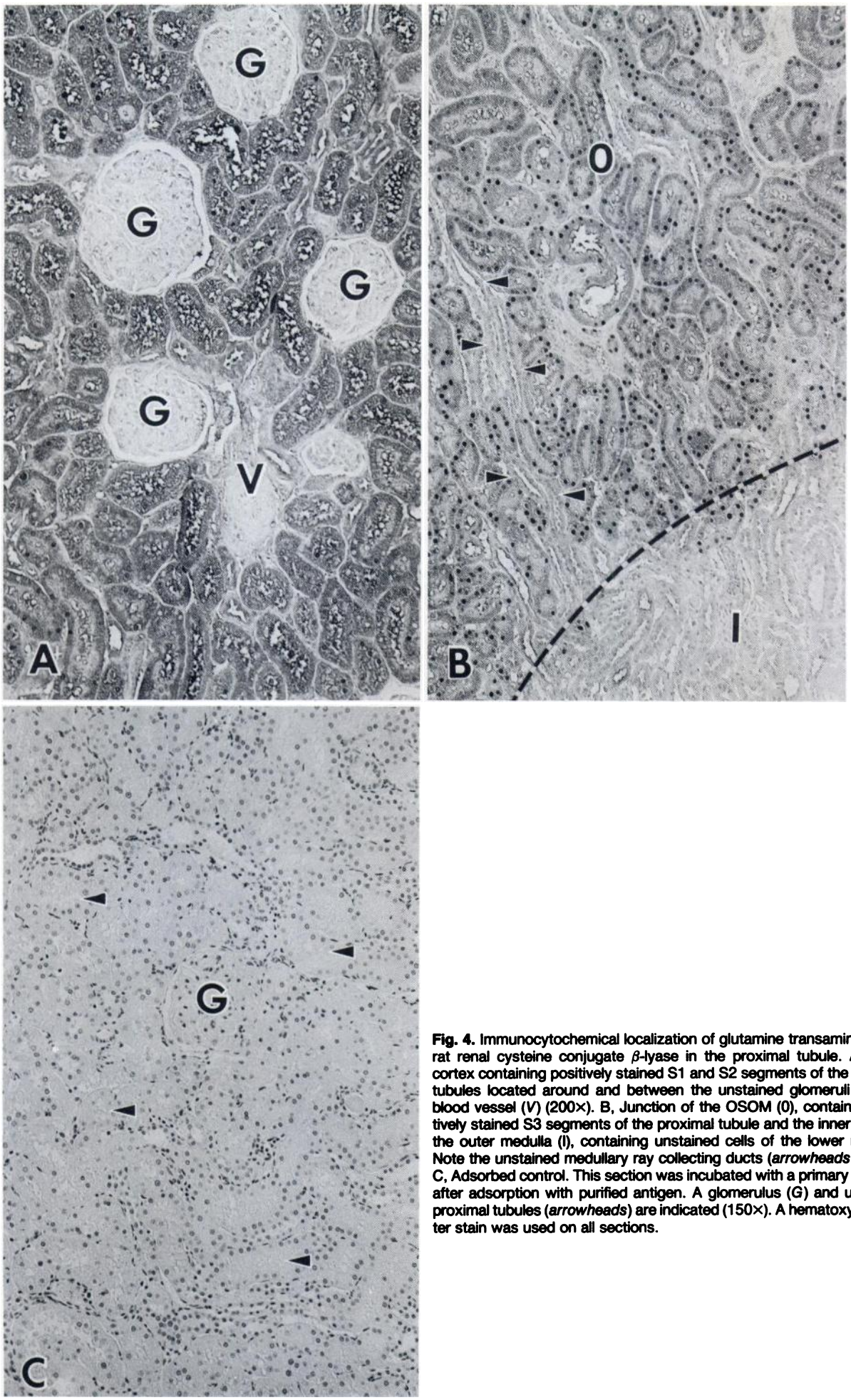

Fig. 4. Immunocytochemical localization of glutamine transaminase K, a rat renal cysteine conjugate β-lyase in the proximal tubule. A, Renal cortex containing positively stained S1 and S2 segments of the proximal tubules located around and between the unstained glomeruli (*G*) and blood vessel (*V*) (200×). B, Junction of the OSOM (0), containing positively stained S3 segments of the proximal tubule and the inner stripe of the outer medulla (I), containing unstained cells of the lower nephron. Note the unstained medullary ray collecting ducts (*arrowheads*) (150×). C, Adsorbed control. This section was incubated with a primary antibody after adsorption with purified antigen. A glomerulus (*G*) and unstained proximal tubules (*arrowheads*) are indicated (150×). A hematoxylin counter stain was used on all sections.

TABLE 1

Tissue distribution of glutamine transaminase K activity and DCVC metabolism (β-Lyase Activity)

	Glutamine Transaminase K	DCVC Metabolism	
		Unstimulated	α-Keto-δ-Methiolbutyrate stimulated
	nmol/min/mg of protein		
Kidney	9.29 ± 2.27	1.32 ± 0.18	3.05 ± 0.27
Liver	1.46 ± 0.24	0.22 ± 0.08	0.84 ± 0.13
Testes	1.55 ± 0.23	0.07 ± 0.01	0.40 ± 0.02
Pancreas	1.11 ± 0.23	0.02 ± 0.01	0.14 ± 0.03
Spleen	1.19 ± 0.20	<0.01	0.23 ± 0.03
Heart	1.12 ± 0.26	<0.02	0.47 ± 0.02
Muscle	1.23 ± 0.22	<0.02	0.44 ± 0.08
Brain	0.82 ± 0.11	<0.02	0.21 ± 0.02
Lung	0.58 ± 0.07	<0.01	0.11 ± 0.02
Bone Marrow	0.48 ± 0.08	<0.01	0.03 ± 0.01

activity with DCVC previously reported (16). However, mitochondria also contain immunoreactive protein, consistent with the presence of glutamine transaminase K in that fraction (15, 20). Taken together, the data in Figs. 1–3 indicate that the antibody recognizes only glutamine transaminase K, a major cytosolic and mitochondrial rat kidney cortex cysteine conjugate β-lyase (5, 15).

The proximal tubule of the rat consists of three distinct segments. The S1 and S2 segments roughly correlate to the pars convoluta, located in the cortex. The pars recta contains the S3 segment, which is located in the medullary rays and the OSOM. When we investigated the intrarenal distribution of β-lyase by immunohistochemistry, the most intense staining involved the proximal tubule. However, peroxidase staining was evenly distributed throughout the cortex and the OSOM, indicating no qualitative differences in the distribution of the enzyme (Fig. 4, A and B). The staining was completely blocked when the primary antibody was preadsorbed with purified antigen (Fig. 4C). Higher dilutions of antibody resulted in weaker staining, but the pattern of intensity did not change along the tubule. Many of the nuclei in the proximal tubular cells located in the medullary rays and OSOM (corresponding to the S3 segment of the proximal tubule) stained highly positive. This appears to be an artifact of ethanol fixation because this was not seen with formalin or Bouin's fixatives (see Experimental Procedures). Cells of the distal tubules and collecting ducts stained much less intensely, if at all, whereas cells of the glomeruli and vascular elements clearly did not stain. The results indicate that the enzyme is located predominantly in the proximal tubular epithelial cells. No gross differences in staining are apparent among the various segments in the cortex and the OSOM.

Although kidney is the primary target of cysteine conjugate toxicity, other organ abnormalities have been observed after administration of DCVC. Parker (21) showed that both kidney and liver mitochondria were affected by DCVC and that incorporation of radiolabeled amino acids into protein was markedly inhibited in the pancreas but not liver and kidney. Therefore, we examined the distribution of the specific activity of glutamine transaminase K and cysteine conjugate β-lyase activity (Table 1). Because we have shown that the β-lyase activity with DCVC can be regulated by the presence of α-keto acids, we determined the DCVC metabolism in the presence and absence of α-keto-δ-methiolbutyrate (Table 1). The mechanism of the regulation by α-keto acids has been discussed previously (Refs. 1 and 5; also see Discussion).

Glutamine transaminase and β-lyase activities were highest in the kidney and the renal activity was stimulated when α-keto-δ-methiolbutyrate was added to the homogenate. Liver has a substantial amount of glutamine transaminase activity, consistent with the data of Cooper and Meister (22) who showed that rat liver contains a glutamine transaminase designated the L-type, as well as smaller amounts of glutamine transaminase K relative to the kidney. Cysteine conjugate β-lyase activity with DCVC was also quite high, compared with some of the other organs, but the ratio of glutamine transaminase to β-lyase activity was less in the liver than in the kidney. This may suggest that less of the activity is contributed by a glutamine transaminase type enzyme, consistent with the previous observation that a portion of β-lyase in rat liver is contributed by the pyridoxal phosphate-dependent enzyme kynureninase (23). Testis, spleen, heart, muscle, and pancreas all had glutamine transaminase activity similar to the activity in liver, but there was less β-lyase activity in these organs than was observed in the liver.

Immunohistochemistry was also used to investigate the tissue distribution of β-lyase. The ethanol-fixed tissues used in this study included liver, pancreas, small and large intestine, lung, testis, spleen, heart, and skeletal muscle. The liver was the only extrarenal tissue to show a significant degree of staining (Fig. 5). The hepatic staining was, however, less intense than that seen in the kidney. The staining appeared to be confined to hepatocytes. The pattern was generally found to be uniform throughout the lobule. No consistent staining patterns were observed in any of the other tissues studies, indicating the limited sensitivity of the immunohistochemical approach.

Discussion

The major renal cysteine conjugate β-lyase, which is active with DCVC, has been shown to be glutamine transaminase K (5, 15). The immunohistochemical (Fig. 4) and metabolism (Table 1) data reported here show that the kidney contains the highest amount of glutamine transaminase K and cysteine conjugate β-lyase activities. Recently, it has been reported that glutamine transaminase K is also active with at least two other nephrotoxic cysteine conjugates (24). The immunohistochemical data reported here are consistent with the proximal tubule specificity of glutathione and cysteine conjugate nephrotoxicity.

The proximal tubule has been shown to consist of distinct segments composed of cells that differ from one another mophologically, functionally, and in their sensitivity to a wide variety of toxins (25, 26). In the case of cysteine conjugate nephrotoxicity, the S3 segment has been shown to be the most sensitive (9–12). This suggests that the cells of the S3 segment are either inherently more sensitive to nephrotoxic cysteine conjugates than the cells of the S1 and S2 segments or that more of the toxic conjugate is activated in S3. The most convincing data favoring the latter explanation are those of Nash *et al.* (9), who showed that bound label from [^{14}C]hexchlorobutadiene is concentrated in the OSOM. However, the immunohistochemical results indicate no marked difference in the distribution of glutamine transaminase K along the proximal tubule. Therefore, we must consider that factors other than the localization of the enzyme may contribute to the sensitivity of the S3 segment.

Because these studies were done at the light microscopic

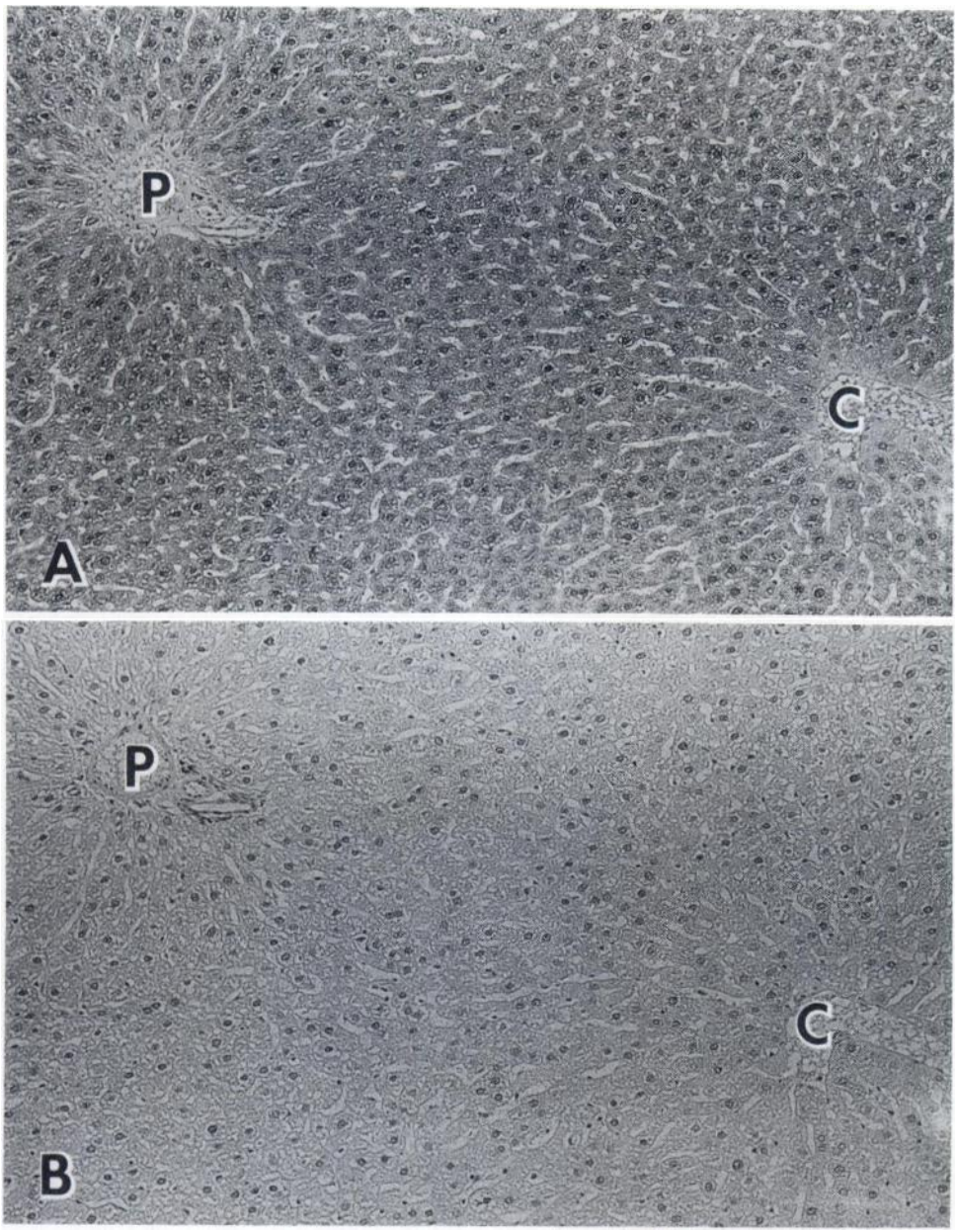

Fig. 5. Immunocytochemical localization of the renal form of the cysteine conjugate β-lyase in rat liver. A, Positive hepatic staining; B, adsorbed control. This serial section was incubated with a primary antibody after adsorption with purified antigen. A portal tract (*P*) and central vein (*C*) are indicated (140×). A hematoxylin counter stain was used on all sections.

level, we cannot exclude the possibility that there is differential distribution of the mitochondrial enzyme along the proximal tubule. Mitochondria appear to be an important target for cysteine conjugate toxicity (27–29). The antibody used in these studies is specific for rat kidney glutamine transaminase K (Figs. 1–3) and recognizes both the mitochondrial and cytosolic isozymes of this enzyme (15). Glutamine transaminase K is responsible for the majority of DCVC metabolism in rat kidney cytosol and mitochondria (5, 15). The relative contributions of glutamine transaminase K in the mitochondrial and cytosolic fractions, which contain the majority of the activity with DCVC (16), is unclear at this time. However, it should be noted that isolated mitochondria are able to activate cysteine conjugates to toxic species (27–29). Additional studies using immunoelectron microscopy might clarify this point.

Immunocytochemical data indicate only the location of a protein in a tissue section, not the activity of that protein. It has been established that cysteine conjugate β-lyase activity is regulated by cellar factors (5, 6, 15). Therefore, it is possible that the *activity* of the enzyme could vary in the tubule segments even though there are no qualitative differences in the distribution. In addition, we cannot exclude the possibility that the reactive metabolite is produced in all segments of the tubule but is concentrated in the S3 segment.

Differences in the entry of mercapturate pathway intermediates into the cells of a particular tubular segment might also contribute to differences in toxicity by regulating the amount of conjugate available for activation. Cysteine conjugate β-lyase is an intracellular enzyme; therefore, transport of the cysteine conjugate (30, 31) or its mercapturate (32, 33) into the cells must occur before activation. It has been suggested that cysteine conjugates enter the tubular epithelium as their *N*-acetyl-L-cysteine derivatives (33) and are deacetylated before activation (34). Furthermore, *N*-acetylcysteine conjugates may be

actively transported by the renal organic anion transporter (32, 33), and probenecid has been shown to block hexachlorobutadiene nephrotoxicity (33). Although reports that organic anion transport is highest in the S2 segment of the rabbit proximal tubule (33) are not consistent with S3 specificity, the deacetylase activity (34) could be higher in S3 than in other segments. Therefore, transport of *N*-acetylcysteine conjugates and their subsequent deacetylation could be important contributors to tubular specificity.

In conclusion, the data presented here suggest that the sensitivity of the S3 segment of the proximal tubule to cysteine conjugate toxicity cannot be explained simply by qualitative differences in the presence of glutamine transaminase K, a major cysteine conjugate β-lyase from rat kidney. Other factors that could play a role include transport, regulation of the rate of metabolism by cellular factors, and differential distribution of mitochondrial β-lyase. Additional studies are necessary to determine whether any of these hypotheses could play a role. However, the ability to measure a physical parameter associated with cellular damage, i.e., covalent binding, may make cysteine conjugates an important tool in understanding the exquisite sensitivity of the proximal straight tubule to a variety of toxic insults.

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