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RENAL TRANSTUBULAR TRANSPORT OF MERCAPTURIC ACID IN VIVO

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Summary

When *S*-benzyl-*N*-acetyl-L-[U-^{14}C]cysteine, a mercapturic acid, was administered to rats intravenously, the plasma level of radioactivity decreased very rapidly with a concomitant increase in the renal level of radioactivity. The renal radioactivity reached its maximum within 2 min and then decreased rapidly with concomitant appearance of the radioactive mercapturic acid in the urine. Bilateral ligation of the ureters resulted in only a slight decrease in the rate of disappearance of mercapturic acid from the plasma, while bilateral nephrectomy caused a marked retardation of its clearance from the plasma. Intravenous administration of probenecid, a well known inhibitor of a renal transtubular transport system for organic acids, caused a significant retardation of mercapturate clearance from the plasma in both of the control and ureter-ligated animals. The renal accumulation of this mercapturic acid as well as its excretion into urine was inhibited by probenecid.

All these data suggested that a mercapturic acid in the plasma was preferentially taken up by renal tubule cells from the basolateral side of plasma membranes via the probenecid-sensitive transtubular transport system and then excreted rapidly into the lumenal space. This transtubular transport of a mercapturic acid seems to constitute an important process in the hepato-renal cooperation in the mercapturic acid biosynthesis in vivo.

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Introduction

It is well known that glutathione plays an important role in non-oxidative detoxication of foreign compounds. The biosynthesis of *N*-acetylcysteine-*S*-conjugates of xenobiotics (mercapturic acids) involves a sequence of diverse reactions [1—5]. Among various mammalian tissues, the conjugation of xenobiotics with glutathione is believed to occur predominantly in the liver, since glutathione *S*-transferases are most abundant in this organ [6,7]. These glutathione *S*-conjugates are hydrolyzed to their component amino acids (glutamic acid, glycine and cysteine-*S*-conjugates) by the cooperative action of γ-glutamyltransferase and some peptidases on the renal brush border membranes or on the lumenal surface of the bile canaliculi and small intestine [8—14]. The cysteine *S*-conjugates thus formed would be acetylated on their α-amino group both in the liver and kidney, which possess a high activity of *N*-acetyltransferase specific for *S*-substituted cysteines [2,15,16]. Then, *N*-acetylcysteine *S*-conjugates are finally excreted, mainly into the urine. Although individual enzymic processes in the mercapturic acid biosynthesis in these tissues have been extensively studied, little is known about the mechanism of potential inter-organ cooperation in processing the individual metabolites and the in vivo mechanism of renal excretion of mercapturic acids, the final metabolites derived from glutathione *S*-conjugates of xenobiotics. The present paper describes evidence for the presence of a renal transtubular transport system for *S*-benzyl-*N*-acetyl-L-cysteine, a mercapturic acid.

Materials and Methods

Materials. Probenecid and sodium hippurate were purchased from Sigma Chemical Co. Heparin and benzyl chloride were obtained from Nakarai Chemicals Co. (Kyoto). L-[U-^{14}C]Cysteine was purchased from The Radiochemical Centre, Amersham (25.1 Ci/mol). Other reagents used were of analytical grade.

Synthesis. *S*-Benzyl-*N*-acetyl-L-[U-^{14}C]cysteine was synthesized from acetic anhydride and *S*-benzyl-L-[U-^{14}C]cysteine by the method of Zbarsky and Young [17]. The specific activity of the product synthesized was $2.0 \cdot 10^4$ cpm/μmol.

Animals and operation. Male Wister rats weighing between 100 and 120 g or between 200 and 220 g were used in the present experiments. They were fasted for 16 h prior to the following operations. Bilateral ligation of the ureter was performed at 0.5 cm distal to the renal pelvis 1 h before experiments. Bilateral nephrectomy was performed 10 min before experiments. Operation was performed by using rats weighing 200—220 g between 9.00 a.m. and 11.00 a.m. All experiments were carried out under pentobarbital anesthesia (50 mg/kg).

Plasma clearance of the mercapturic acid. At 5 min before experiments, animals were heparinized intravenously (2500 units/kg). *S*-Benzyl-*N*-acetyl-L-[U-^{14}C]cysteine (4 mM) dissolved in 0.15 M NaCl was administered intravenously from the right femoral vein (10 μmol/kg) over a period of 3 s. At timed intervals, 0.1 ml blood samples were collected from the left femoral vein unless otherwise stated. Blood samples were centrifuged at 12 000 rev./min for 3 min in an Eppendorf Centrifuge-5412. Plasma samples thus obtained were analyzed

for their radioactivity. Administration of probenecid (350 μmol/kg) was performed at 3 min before injection of the radioactive mercapturic acid.

Radioactive materials in the liver, kidney and urine. At varying times after administration of the radioactive mercapturic acid, animals were killed by bleeding from the left femoral artery. The liver and kidneys were simultaneously perfused from the abdominal vein with 5 ml of 0.15 M NaCl. The excised liver and kidneys were homogenized separately with 3 vol. of 5% trichloroacetic acid. They were centrifuged at 15 000 × *g* for 10 min. Precipitates were resuspended in 3 vols of 5% trichloroacetic acid, followed by centrifugation at 15 000 × *g* for 10 min. The combined supernatant fractions were analyzed for their radioactivity. Urine samples were obtained by puncture of the urinary bladder and were determined for their radioactivity. Analysis of the radioactive compounds in the serum, liver, kidney and urine was carried out with the acid-soluble fractions essentially as described by Green and Elce [15]. Measurement of radioactivity was carried out in a Tri-Carb scintillation spectrometer 3320 with Bray's solution as a scintillant.

Results

Distribution of intravenously administered S-benzyl-N-acetyl-L-[U-^{14}C]cysteine

S-Benzyl-*N*-acetyl-L-[U-^{14}C]cysteine was administered intravenously to rats and the level of radioactivity was determined on the serum, kidney, liver and urine at timed intervals (Fig. 1). The serum level of radioactivity decreased very rapidly during the first few minutes of administration with a concomitant increase in the renal level of radioactivity. The renal level reached its maximum within 2 min of administration, and then decreased rapidly. Concomitant with

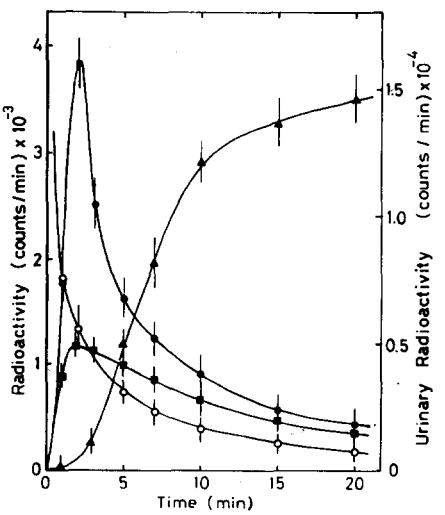

Fig. 1. Time course of variations in radioactivity derived from *S*-benzyl-*N*-acetyl-L-[U-^{14}C]cysteine in the serum, kidney, liver and urine. After intravenous administration of the radioactive mercapturic acid (10 μmol/kg), the amounts of radioactivity in the plasma, kidney, liver and urine were determined at the indicated times. Experiments were performed by using rats weighing 100—120 g. Details of sampling procedures were as described under Materials and Methods. Each point represents the mean ± S.D. of triplicate experiments. ○, plasma; ●, kidney; ■, liver; ▲, urine.

this rapid decrease in the renal level, a significant amount of radioactivity appeared in the urine. The initial accumulation of radioactivity in the liver was much less marked. Chemical analysis of the radioactive compounds in the serum, kidney, liver and urine revealed that more than 96% of the radioactivity was accounted for by the intact mercapturic acid (data not shown). These results suggested that the mercapturic acid in the plasma was preferentially extracted by the kidney and excreted into urine very rapidly. This is consistent with the previous observation [14] that an *S*-substituted derivative of *N*-acetylcysteine intravenously administered to mice was accumulated in the kidney and then excreted into urine.

Plasma clearance of the mercapturic acid. To know the mechanism of renal disposition of mercapturic acids, the plasma clearance of *S*-benzyl-*N*-acetyl-L-[U-^{14}C]cysteine was examined in rats previously subjected to either nephrectomy or ureter ligation. The plasma level of radioactivity in the control animals decreased rapidly after intravenous administration of the mercapturic acid (Fig. 2). The rate of disappearance of radioactivity markedly decreased after bilateral nephrectomy, indicating a significant participation of the kidney in the plasma clearance of this mercapturic acid. Ligation of the ureter has been used to examine whether or not the renal disposition of circulating compounds depends on its glomerular filtration. In contrast to the nephrectomized rats, no significant decrease in the rate of plasma clearance of this mercapturic acid was observed with the ureter-ligated animals (Fig. 3). This finding can be interpreted as showing that a major part of this mercapturic acid in the plasma was taken up by the kidney via an extraglomerular transport system.

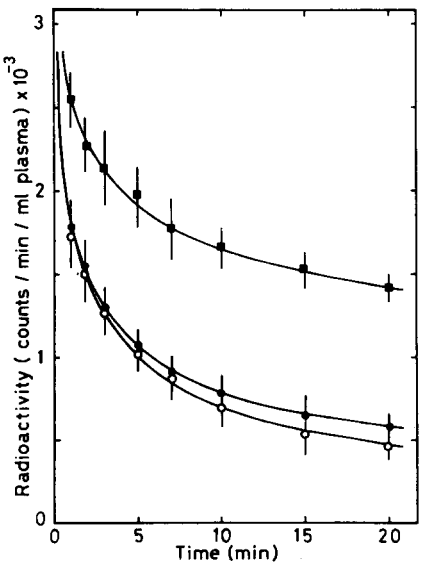

Fig. 2. Plasma clearance of *S*-benzyl-*N*-acetyl-L-[U-^{14}C]cysteine. After intravenous administration of the radioactive mercapturic acid (10 μmol/kg), the level of radioactivity in the plasma was determined with the control (○), ureter-ligated (●) and nephrectomized animals (■). Experiments were performed by using rats weighing 200—220 g. Details were as described under Materials and Methods.

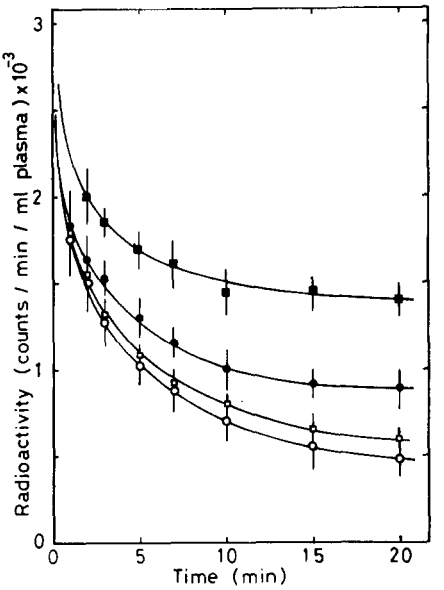

Fig. 3. Effect of probenecid on the plasma clearance. Effect of intravenous administration of probenecid on the plasma clearance of *S*-benzyl-*N*-acetyl-L-[U-^{14}C]cysteine was observed in the control (○) and ureter-ligated animals (squares) in the absence (open symbols) and presence of probenecid (closed symbols). Administration of probenecid (350 μmol/kg) was performed 3 min before injection of the radioactive mercapturic acid (10 μmol/kg). Other conditions were the same as in Fig. 2.

Effect of probenecid on the plasma clearance. *N*-acetylated cysteine-*S*-conjugates are organic acids in nature. Like hippuric acid, many organic acids have been known to receive renal transtubular secretion [18,19]. Probenecid is widely employed as a specific inhibitor of this transtubular transport system [19—21]. To test whether or not mercapturic acids are also excreted into urine via this transport system, the effect of probenecid on the plasma clearance of the mercapturic acid was examined (Fig. 3).

The rate of plasma clearance of the mercapturic acid markedly decreased in animals which were previously administered probenecid. The effect of pro-

TABLE I

EFFECT OF PROBENECID PRETREATMENT ON THE RENAL ACCUMULATION AND URINARY EXCRETION OF THE MERCAPTURIC ACID

Amounts of radioactivity in the kidney and urine were determined on the control and probenecid-treated animals after 20 min of intravenous administration of *S*-benzyl-*N*-acetyl-L-[U-^{14}C]cysteine (10 μmol/kg). Other conditions were the same as in Fig. 3.

Animals	Probenecid	Radioactivity (cpm)	
		Kidney	Urine
Control	0	2420 ± 840	18 600 ± 2400
	350 μmol/kg	750 ± 110	5800 ± 600
Ureter-ligated	0	7050 ± 680	—
	350 μmol/kg	1250 ± 150	—

benecid in retarding the plasma clearance of the mercapturic acid was much more marked with the ureter-ligated animals than with the control animals. A similar retarding effect on the plasma clearance was observed by administration of hippuric acid (data not shown). These data clearly indicated that a significant part of this mercapturic acid in the plasma was taken up by the renal tubule cells from the basolateral side of plasma membranes via the probenecid-sensitive organic anion transport system.

Approx. 50% of the injected radioactivity was found to appear in the urine of control rats within 20 min of administration, while only 17% was excreted into urine collected from probenecid-treated rats (Table I). The renal level of radioactivity was also found to decrease by treatment with probenecid. However, the renal level was significantly lower than that of the urinary level in either experiment. The renal level of radioactivity in ureter-ligated rats was higher than that in the control animals and this renal accumulation was also inhibited by probenecid.

Discussion

The present experiments demonstrated that *S*-benzyl-*N*-acetylcysteine in the plasma was preferentially taken up by renal cells and excreted into urine very rapidly. Although the subcellular localization of this transport system is not clear at present, several lines of evidence presented in this investigation are consistent with the contention that the mercapturic acid is processed via a probenecid-sensitive transtubular transport system which has been thought to be localized on the basolateral membranes of the proximal tubules [19—21].

No significant part of the renal extraction of this mercapturic acid seems to occur by its glomerular filtration, since the plasma clearance was not affected by ureter ligation. In contrast, preliminary experiments revealed that a significant part of *S*-carbamidomethyl-*N*-acetylcysteine in the plasma was extracted by renal glomerular filtration. We speculate that the relative ratio of the tubular transport to the glomerular filtration in the renal extraction of a mercapturic acid may depend on the structural feature of its *S*-substituent. Further quantitative study in this aspect should be important in assessing the general feature of processing of mercapturic acids having various xenobiotics as their *S*-substituent.

A major part of the administered radioactivity (50—75%) was found to be excreted into the urine within 20 min of administration. More than 98% of the radioactivity in the urine samples was accounted for by the intact form of this mercapturic acid in either experiment with the control and probenecid-treated animals (data not shown). This is in good agreement with the report [22] that *S*-benzyl-*N*-acetylcysteine administered to rats by stomach tube was excreted into urine mainly as the intact form.

Preliminary experiments in vivo using a liver perfusion technique revealed that *S*-benzyl-L-cysteine was actively taken up by the cells, converted to its *N*-acetyl derivative, and then released rapidly into the effluent perfusate as the mercapturic acid. The mercapturic acid thus formed in the liver cells would be transferred to the kidney via the blood circulation and excreted into urine predominantly via a probenecid-sensitive transtubular transport system in the renal

proximal tubules. The inhibitory action of probenecid might be due to the competition for the cellular uptake process of the mercapturic acid, since the renal accumulation of this mercapturic acid was also inhibited by probenecid with ureter-ligated animals (Table I). Preliminary experiments using isolated renal proximal tubules revealed that the uptake of this mercapturic acid from the basolateral surface membranes was competitively inhibited by probenecid. However, the mechanism of the rapid export of the intracellular mercapturic acid into the tubular lumenal space remains to be studied.

A recent report [23] showed that the renal extraction of cyclic AMP was apparently inhibited by probenecid. This inhibition was ascribed to the decrease in the accumulation of metabolites of cyclic AMP which occurred secondary to the inhibition of phosphodiesterase activity by probenecid. Thus, it remains also possible that some metabolic perturbations induced by probenecid may result in the retardation of the plasma clearance and urinary excretion of this mercapturic acid.

References

1 Bray, H.G., Franklin, T.J. and James, S.P. (1959) Biochem. J. 71, 690—696
2 Bray, H.G., Franklin, T.J. and James, S.P. (1959) Biochem. J. 73, 465—473
3 Boyland, E. and Chasseaud, L.F. (1969) Adv. Enzymol. 32, 173—219
4 Wood, J.L. (1970) in Metabolic Conjugation and Metabolic Hydrolysis (Fishman, W.H., ed.), pp. 261—299, Academic Press, New York
5 Chasseaud, L.F. (1976) in Glutathione: Metabolism and Function (Arias, I.M. and Jakoby, W.B., eds.), pp. 77—114, Raven Press, New York
6 Habig, W.H., Pabst, M.J. and Jakoby, W.B. (1974) J. Biol. Chem. 249, 7130—7139
7 Jakoby, W.B. (1978) in Functions of Glutathione in Liver and Kidney (Sies, H. and Wendel, A., eds.), pp. 157—163, Springer-Verlag, Berlin
8 Binkley, F. (1961) J. Biol. Chem. 236, 1075—1082
9 Glossman, H. and Neville, D.M., Jr. (1972) FEBS Lett. 19, 340—344
10 Robinson, D.S., Birnbaum, S.M. and Greenstein, J.P. (1953) J. Biol. Chem. 202, 1—26
11 Semenza, G. (1957) Biochim. Biophys. Acta 24, 401—413
12 Silbernagl, S., Pfaller, W., Heinle, H. and Wendel, A. (1978) in Function of Glutathione in Liver and Kidney (Sies, H. and Wendel, A., eds.), pp. 60—69, Springer-Verlag, Berlin
13 Curthoys, N.P. and Hughey, P. (1979) Enzyme 24, 383—403
14 Inoue, M., Okajima, K., Ito, K., Horiuchi, S. and Morino, Y. (1979) XIth Int. Congrr. Biochem., Toronto, p. 470
15 Green, R.M. and Elce, J.S. (1975) Biochem. J. 147, 283—289
16 Hughey, P. and Curthoys, N.P. (1978) Arch. Biochem. Biophys. 186, 211—217
17 Zbarsky, S.H. and Young, L. (1943) J. Biol. Chem. 151, 211—215
18 Marshall, E.K. and Crane, M.M. (1924) Am. J. Physiol. 70, 465—488
19 Weiner, M.I. and Mudge, G.H. (1964) Am. J. Med. 36, 743—762
20 Weiner, I.M., Washington, J.A. and Mudge, G.H. (1960) Bull. Johns Hopkins Hosp. 106, 333—346
21 Dayton, P.G., Yü, T.F., Chen, W., Berger, L., West, L.A. and Gutman, A.B. (1963) J. Pharmacol. Exp. Ther. 140, 278—286
22 Bray, H.G. and James, S.P. (1960) Biochem. J. 74, 394—397
23 Podevin, R.A., Boumendil-Podevin, E.F., Bujoli-Roche, J. and Priol, C. (1980) Biochim. Biophys. Acta 629, 135—142