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PARTICIPATION OF PEROXISOMAL β-OXIDATION SYSTEM IN THE CHAIN-SHORTENING OF A XENOBIOTIC ACYL COMPOUND

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A drug, (E)-3-[4-(1-imidazolylmethyl)phenyl]-2-propenoic acid, was metabolized to 4-(1-imidazolylmethyl)benzoic acid in isolated hepatocytes of rats, which was enhanced markedly by the pretreatment of rats with clofibrate. With liver homogenates, the formation of the CoA-ester of this drug and its subsequent chain-shortening were demonstrated. In the series of these reactions, acyl-CoA synthetase, CoA, ATP and NAD were required, whereas cyanide did not inhibit the reaction. These results indicate that peroxisomes are capable of shortening the acyl side-chains of drugs by the β-oxidation, giving an additional suggestion on the functions of peroxisomes.

Some drugs having acyl side-chains in their chemical structures have been known to be metabolized oxidatively to give the chain-shortened products, and this reaction has been considered to be catalyzed by the mitochondrial β-oxidation. Recently, a fatty acid oxidizing system, different from the mitochondrial system, was discovered in peroxisomes (1). This system was found to oxidize very long chain-trans-monounsaturated fatty acids, known to be poor substrates for the mitochondrial β-oxidation, at a similar rate to those of the corresponding cis-isomers (2). In addition, it was also found that peroxisomal β-oxidation was involved in the conversion of 3α,7α,12α-trihydroxy-5β-cholestanoic acid to cholic acid (3) and in the cleavage of cholesterol side-chain (4). Thus, in terms of the substrate specificity of peroxisomal β-oxidizing system and the chemical structures of drugs, this system is possible to take part in the metabolism of such drugs. In this paper, peroxisomal β-oxidation of the

The abbreviations used; IMPP, (E)-3-[4-(1-imidazolylmethyl)phenyl]-2-propenoic acid. IMB, 4-(1-imidazolylmethyl)benzoic acid.

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acyl side-chain of a drug is described, using (E)-3-[4-(1-imidazolylmethyl) phenyl]-2-propenoic acid (IMPP) as a model drug.

MATERIALS AND METHODS

ANIMALS AND TREATMENT; Male Wistar rats weighing about 150g were kept on a regular chow diet with or without 0.25% clofibrate for 2 weeks.

EXPERIMENTS WITH ISOLATED HEPATOCYTES; Hepatocytes were prepared by collagenase-perfusion method according to Moldeus et al. (5). IMB-formation from IMPP was measured as follows. The mixture containing $1x10^6$cells of hepatocytes and 2.5mM [^{14}C]IMPP (2.2 μCi) in a final volume of 0.5ml of Krebs-Henseleit buffer (pH 7.4) was incubated at 37°C, and the aliquots of 50μl were withdrawn at various time-intervals and added to aqueous NaOH (0.1N in the final) containing non-labelled IMB as the carrier. After the hydrolysis for 30min at 55°C, the mixtures were acidified by HCl and were subjected to TLC with $CHCl_3$-CH_3OH-H_2O (65:25:3, by vol.). The bands corresponding to the Rf. values of IMB and others were scraped into counting vials containing 0.5ml of 0.5N HCl to solubilize the metabolites and, after the addition of the scintillation fluid, the radioactivities were measured in an Aloka model 903 liquid scintillation counter.

SUBCELLULAR FRACTIONATION; Liver homogenates (10%, w/v) were prepared in 0.25M sucrose containing 20mM glycylglycine (pH 7.4), and differential centrifugation was carried out according to the method of De Duve et al. (6). The pellet from each step was suspended in the above solution. The light mitochondrial (L) fraction was further centrifuged on a discontinuous sucrose density gradient as described previously (7).

ENZYME ASSAYS; The activities of cyanide-insensitive fatty acyl-CoA oxidizing system, carnitine palmitoyltransferase, catalase, cytochrome c oxidase and glucose-6-phosphatase were determined as described previously (7,8). Protein concentration was determined by the method of Lowly et al. (9).

IMPP-CoA ESTER FORMATION; The mixture containing 2.5mM [^{14}C]IMPP (0.2μCi), 2.5mM CoA, 5.0mM ATP, 5.0mM $MgCl_2$, 0.3mg/ml defatted bovine serum albumin, 60mM potassium phosphate (pH 7.4) and an enzyme preparation (approximately 20μg protein of liver homogenate) in a final volume of 50μl was incubated at 37°C and the aliquots of 5μl were withdrawn at various time-intervals and applied directly on thin-layer. After development, the radioactivity of the band corresponding to IMPP-CoA ester was measured as described above. For product identification the aqueous extract obtained from the band was hydrolyzed, and then devided to CoA and IMPP by TLC.

IMB-FORMATION FROM IMPP-CoA ESTER; IMPP-CoA ester was prepared by incubating the mixture of IMPP, commercial acyl-CoA synthetase (Sigma Co.) and others as described above. This was incubated with enzyme preparation, NAD (1mM), KCN (1mM), Triton X-100 (0.01%) and potassium phosphate buffer (30mM, pH 7.4) in a final volume of 100 μl for 60 min. The enzyme preparations used were liver homogenate, L-fraction from differential centrifugation and peroxisomal fraction from sucrose density gradient centrifugation. The reaction was terminated by the addition of aqueous NaOH containing non-labelled IMB, and aliquots of the reaction mixture were subjected to the analysis of IMB-formation by TLC method described above.

MATERIALS; (E)-3-[4-(1-Imidazolyl-[^{14}C]methyl)phenyl]-2-propenoic acid (IMPP) and IMB were kindly donated by Central Research Laboratory, Kissei Pharmaceutical Co.,Ltd.,.

RESULTS AND DISCUSSION

When IMPP was administered to rats orally (30 mg/kg), more than 90% of dose was excreted into the urine and feces within 24 h, and the major

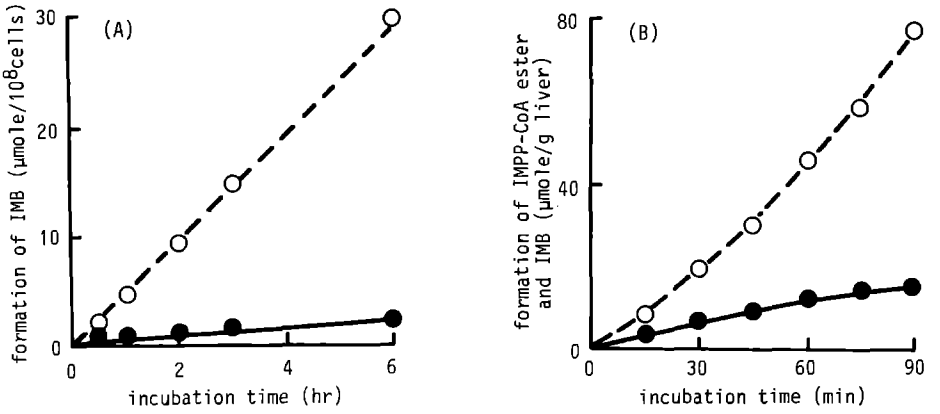

Fig.1. (A); Formation of IMB from IMPP in isolated hepatocytes from control (solid lines) and clofibrate-treated (dotted lines) rats. (B); Formation of IMB (solid lines) and IMPP-CoA ester (dotted lines) in liver homogenates from clofibrate-treated rats. Incubations were carried out as described under MATERIALS AND METHODS.

metabolite was IMB having a chemical structure two-carbon unit less than in the acyl chain IMPP-molecule. On the other hand no metabolite with less one-carbon unit was detected. Thus it is suggested that β-oxidation participates in the metabolism of the acyl side-chain of IMPP.

As shown in Fig.1, the conversion of IMPP to IMB apparently occurred in isolated hepatocytes of rats and the rate of IMB-formation was enhanced markedly by the pretreatment of rats with clofibrate. Lazarow and De Duve (1) have shown the enhancement of hepatic peroxisomal β-oxidation toward fatty acyl-CoA in the rat treated with clofibrate. When the liver homogenates were used, the IMB-formation proceeded with incubation time under the conditions of peroxisomal β-oxidation reaction in the presence of cyanide, and then the activity of the formation of IMPP-CoA ester was much higher than above activity. These results suggest that the peroxisomal β-oxidation is involved in the reaction and that the activation of IMPP is not a rate limiting in the rat liver. Table I indicates that the formation of IMPP-CoA ester is essential as an intermediary step, since this cyanide-insensitive IMB-formation depends on acyl-CoA synthetase, CoA and ATP. Moreover, the dependence of the reaction on NAD indicates the participation of β-oxidation in the IMB-formation from IMPP-CoA. When the cyanide,

Table I Cofactor requirement for cyanide-insensitive IMB-formation by the light mitochondrial fraction from the livers of clofibrate-treated rats

	Specific activity (U/mg protein)
Complete assay system	2.31
- Acyl-CoA synthetase	1.12
- CoA	0
- ATP	0.16
- NAD	0
- KCN	3.34

a potent inhibitor for mitochondrial β-oxidation, was omitted from the reaction mixture, the activity increased by about 50% compared with complete system. Thus, it seems that the mitochondrial β-oxidation can also be concerned with IMB-formatation.

The subcellular distribution of the activity of the cyanide-insensitive IMB-formation in the rat liver was illustrated in Fig.2. The activity was exclusively seen in the light mitochondrial fraction, and the distribution pattern was similar to those of marker enzymes for peroxisomes, such as catalase and cyanide-insensitive fatty acyl-CoA oxidizing system. The distribution of this activity was further confirmed by the subsequent fractionation of the light mitochondrial fraction in sucrose density gradient. When this activity was estimated to the homogenate levels, it was sufficient to account for the rate of IMB-formation in isolated hepatocytes and the urinary excretion of this metabolite.

These results indicate that, in the liver of rats, IMPP is activated to the IMPP-CoA ester in a similar manner to fatty acids and then converted to IMB by β-oxidation and that peroxisomes have a potency to metabolize the acyl side-chain of this drug. Since peroxisomal β-oxidation is not directly linked to oxidative phosphorylation, it appears less effective as an energy generating system. It has been pointed out that one of the physiological significances of peroxisomal β-oxidation is the chain shortening of long chain fatty acids to produce better substrates for mitochondrial β-

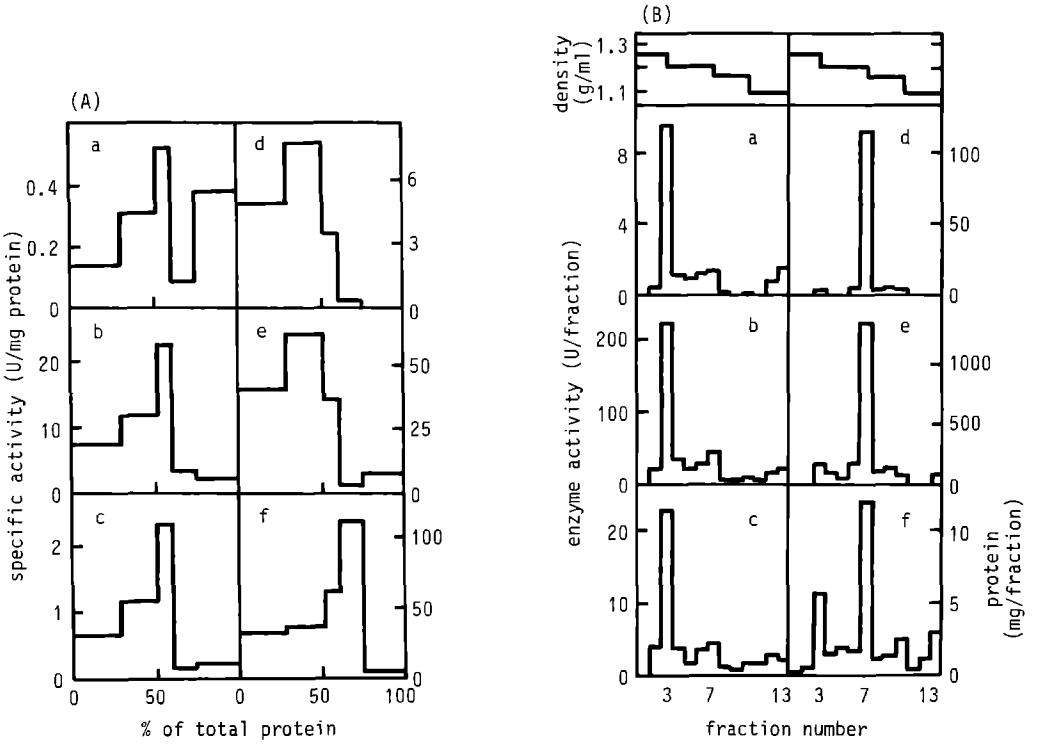

Fig.2. (A); Subcellular distribution of enzyme activities after differential centrifugation of liver homogenates from clofibrate-treated rats. Columns represent, from left to right, nuclear, heavy mitochondrial, light mitochondrial, microsomal and supernatant. a: catalase, b: cyanide-insensitive fatty acyl-CoA oxidizing system, c: cyanide-insensitive IMB-formation, d: cytochrome c oxidase, e: carnitine palmitoyltransferase, f: glucose-6-phosphatase. The units of the above enzyme activities were expressed as nmols of substrates or cofactors transformed per min, except for both a and d which were as in (7). (B); Subfractionation of the light mitochondrial fraction by sucrose density gradient centrifugation. a-e indicate the same enzyme activities as above, and f: protein.

oxidation, when the utilization of fatty acids are stimulated largely as an energy source in starvation (10), feeding high-fat diet (2,11), diabetes (12) or birth and development (13,14). In the present study, our findings give an additional suggestion that peroxisomes play an important role for the metabolism of xenobiotic acyl compounds. Recently, Caldwell (15) mentioned that xenobiotic acyl-CoA species likely play a critical role in the metabolism, pharmacology and toxicity of a wide variety of xenobiotic carboxylic acids. Therefore, further attentions need to be payed about the relationship between peroxisomal β-oxidation and the metabolism of xenobiotic acyl compounds in the body.

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