Biochemical Pharmacology, Vol. 41, No. 4, pp. 617–623, 1991.
Printed in Great Britain.

0006-2952/91 $3.00 + 0.00

CHARACTERISTICS OF INDUCTION OF PEROXISOMAL FATTY ACID OXIDATION-RELATED ENZYMES IN RAT LIVER BY DRUGS

RELATIONSHIPS BETWEEN STRUCTURE AND INDUCING ACTIVITY

HIROKO KOZUKA, JUNJI YAMADA, SHUICHI HORIE, TAKAFUMI WATANABE,* TETSUYA SUGA and TOSHIHIKO IKEDA†

Department of Clinical Biochemistry, Tokyo College of Pharmacy, Tokyo 192–03, Japan; and †Analytical and Metabolic Research Laboratories, Sankyo Co., Ltd., Tokyo 104, Japan

(*Received* 19 *July* 1989; *accepted* 7 *September* 1990)

Abstract—To clarify the mechanism of induction of hepatic peroxisome-associated enzymes by drugs, we examined the interrelationship between the structures of fifteen drugs of two types (phenoxyacetic acid derivatives and perfluorinated compounds) and their inducing activities. Male Wistar rats were given the drugs at 150 mg/kg body weight daily for 2 weeks, and then hepatic activities of fatty acid metabolism-related enzymes were determined. The activity of the cyanide-insensitive fatty acyl-CoA oxidizing system located in peroxisomes was increased significantly in the following order: 2,4,5-trichlorophenoxypropionic acid (12.5-fold) > 2,4-dichlorophenoxypropionic acid (6.6-fold) > clofibrate (4.5-fold) > 2-methyl-4-chlorophenoxyacetic acid (2.6-fold) > 2,4,5-trichlorophenoxyacetic acid (2.5-fold) > *p*-chlorophenoxypropionic acid (2.4-fold) > 2,4-dichlorophenoxyacetic acid (1.7-fold). Treatment with perfluorinated compounds, perfluorobutyric acid, perfluorooctanoic acid, perfluorodecanoic acid and perfluorooctanol, also induced the activity by 2-, 4.3-, 3.1- and 2.0-fold respectively. The profile of the induction of carnitine acetyltransferase by these compounds was quite similar to that of cyanide-insensitive fatty acyl-CoA oxidizing system. Lipophilicity of these drugs was determined by the octanol–water partition method. Among these drugs, 2,4,5-trichlorophenoxypropionic acid showed the largest octanol/water partition coefficient (log P = 0.39). These results show a strong correlation among the number of chlor-substitutions on the phenyl moiety, the methyl-group on the alpha position of the acetic acid moiety, lipophilicity and the inducibility of peroxisomal fatty acid oxidation-related enzymes.

Hypolipidemic drugs, such as clofibrate [1, 2], fenofibrate [3], nafenopin [4], Wy 14,643 [5] and tibric acid [6], induce marked changes in the livers of rats and mice. The initial hepatic responses in rodents after the administration of these peroxisome proliferators are hepatomegaly and marked proliferation of peroxisomes in association with changes in peroxisomal structure and its enzyme composition [7, 8]. These drugs induce hepatocellular tumors after long-term administration in rats and mice [9–11], and Reddy and Rao [12] hypothesized that hepatocarcinomas induced by peroxisome proliferators were due to the oxidative stress caused by enhanced hydrogen peroxide content in association with an increase in peroxisomal fatty acid oxidation [13]. Although possible explanations for the induction of peroxisomal enzymes have been proposed [14] [(1) presence of a drug receptor, (2) relation to a second messenger, and (3) levels of cofactors in the liver], the mechanism by which peroxisome proliferators elicit their pleiotropic responses and the interrelationship between inducing activity and structures of peroxisome proliferators have not yet been elucidated.

In this study we examined the *in vivo* effects of phenoxyacetic acid (PAA‡) derivatives, phenoxypropionic acid derivatives and perfluorinated compounds (see Fig. 1) on peroxisomal fatty acid oxidation-related enzymes in rat liver, and discuss the interrelationship between the structures of drugs and their inducing activities. Furthermore, we also determined the partition coefficients of the compounds, and discuss the interrelationship between lipophilicity and inducing activities of these compounds.

MATERIALS AND METHODS

Materials. Phenoxyacetic acid derivatives and phenoxypropionic acid derivatives were obtained from the Wako Pure Chemicals Co. Ltd. (Japan). Perfluorinated compounds were provided by the Sankyo Co. Ltd. (Japan). L-Carnitine-HCl

* Correspondence: Dr. Takafumi Watanabe, Department of Clinical Biochemistry, Tokyo College of Pharmacy, 1432-1 Horinouchi, Hachioji, Tokyo 192-03, Japan.

‡ Abbreviations: PAA, phenoxyacetic acid; o-CPA, *o*-chlorophenoxyacetic acid; p-CPA, *p*-chlorophenoxyacetic acid; 2,4-D, 2,4-dichlorophenoxyacetic acid; 2,4,5-T, 2,4,5-trichlorophenoxyacetic acid; p-CPP, *p*-chlorophenoxypropionic acid; 2,4-DP, 2,4-dichlorophenoxypropionic acid; 2,4,5-TP, 2,4,5-trichlorophenoxypropionic acid; MCPA, 2-methyl-4-chlorophenoxyacetic acid; CPIB, clofibrate; PFBA, perfluorobutyric acid; PFOA, perfluorooctanoic acid; PFDA, perfluorodecanoic acid; PFOL, 1-*H*,1-*H*-pentadecafluorooctanol; PFOS, perfluorooctanesulfonic acid; DAAO, D-amino acid oxidase; FAOS, cyanide-insensitive fatty acyl-CoA oxidizing system; CAT, carnitine acetyltransferase; CPT, carnitine palmitoyltransferase; and FADH, fatty acyl-CoA dehydrogenase.

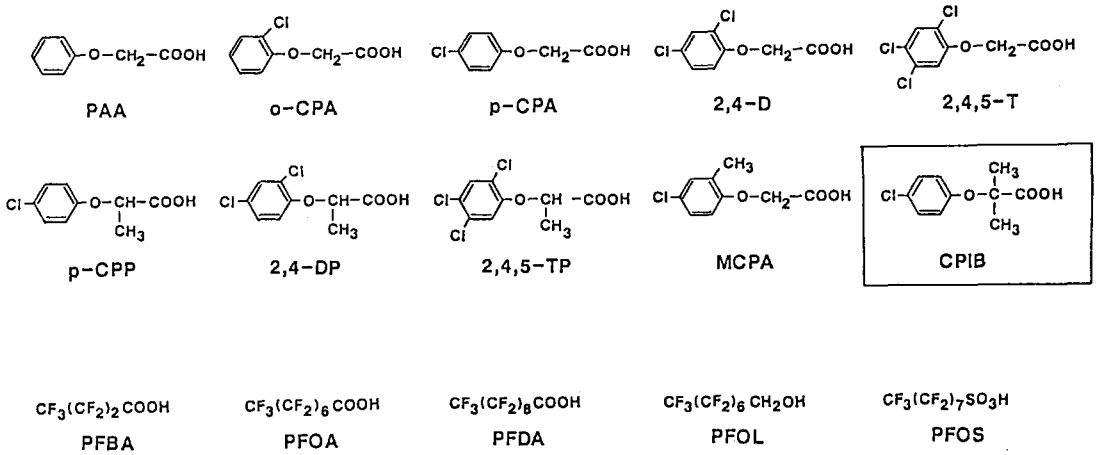

Fig. 1. Chemical structures of phenoxyacetate-derivatives and perfluorinated compounds.

was provided by the Earth Pharmaceutical Co. Ltd. (Japan). Acetyl-CoA, NAD, palmitoyl-CoA and bovine serum albumin (BSA, fatty acid free) were obtained from the Sigma Chemical Co., St. Louis, MO, U.S.A. Other chemicals, all of reagent grade, were obtained from the Wako Pure Chemicals Co. Ltd. (Japan).

Animals and treatment. Male Wistar rats weighing around 180 g were used. These animals were obtained from the Tokyo Laboratory Animals Co. Ltd., Japan. The animals (five animals/group) were given, by oral route, agents other than perfluorinated compounds suspended in 1% methylcellulose at a dose level of 150 mg/kg body weight/day for 2 weeks. Perfluorinated compounds other than perfluorooctanesulfonic acid (PFOS) were solubilized in corn oil and administered intraperitoneally to the rats in a single injection at a dose level of 50 mg/kg body weight, and the animals were maintained for 3 days. PFOS was solubilized in 0.9% NaCl and administered to the rats in the same manner as the other perfluorinated compounds. The animals were weighed and decapitated after the treatments. The livers were perfused and quickly removed, and 10% (w/v) homogenates were prepared in 0.25 M sucrose. The homogenates were used for the following assays.

Assay methods. The activity of cyanide-insensitive fatty acyl-CoA oxidizing system (FAOS) was determined by measuring the palmitoyl-CoA-dependent reduction of NAD at 340 nm by the method described by Lazarow and de Duve [15]. One unit of activity was defined as the amount of the enzyme that reduced 1 nmol/min. The activities of carnitine acetyltransferase (CAT) and carnitine palmitoyltransferase (CPT) were determined spectrophotometrically by measuring the amount of CoA-SH released during the reaction with 5,5′-dithio-bis(2-nitrobenzoic acid) at 412 nm [16]. One unit of both enzyme activities was defined as the amount of the enzyme that produced 1 nmol of CoA-SH from acetyl-CoA or palmitoyl-CoA per min. FADH was determined by the method of Hryb and Hogg [17] using palmitoyl-CoA as substrate, and one unit of the enzyme was defined as the amount of the enzyme that reduced 1 nmol of 2,6-dichlorophenolindophenol per min. Catalase was determined as described in the previous report [18]. A unit of enzyme activity of catalase was defined as the amount of the enzyme giving $K = 1$, where K is the rate constant of the enzyme. D-Amino acid oxidase (DAAO) and urate oxidase were also determined as described in the previous report [18]. Protein content was determined by the method of Lowry *et al.* [19] with BSA as a standard. Statistical evaluations were performed by Student's *t*-test.

Determination of partition coefficients of the compounds. The partition coefficients of the compounds were determined by the flask-shaking method [20]. Briefly, each compound was solubilized in 20 mL of octanol at 1 mg/mL, mixed with the same volume of 300 mM potassium phosphate buffer (pH 7.4), and then the mixture was shaken vigorously for 30 min. After centrifugation, the concentration of the compound in aqueous and octanol phases was determined. The partition coefficient was represented as log (C_0/C_a), where C_0 was the concentration of the compound in the octanol phase and C_a was that in the aqueous phase.

RESULTS

Table 1 shows some biochemical values and the activities of some hepatic peroxisome- and mitochondrion-associated enzymes of control animals. The effects of drugs on these biochemical values and hepatic enzymes are summarized in Tables 2–4. The structural formulas of the drugs used in the present study are shown in Fig. 1, and the drugs are classified into three groups. The first group comprises the phenoxyacetic acid derivatives, which have a varying number of chlor-substitutions at various positions on the phenyl group (Table 2). The second group comprises phenoxypropionic acid derivatives modified with a methyl-group at the alpha-position (Table 3). The third group comprises the perfluorinated compounds (Table 4). There were no significant differences in the body weight gains

Table 1. Liver weight, protein content and activities of some mitochondrial and peroxisomal enzymes of control animals

Parameters	Control value
Relative liver weight (% of body wt)	4.52 ± 0.28
Protein (mg/g liver)	209 ± 5
Activities (units/g liver)	
Catalase	39.9 ± 5.3
DAAO	0.78 ± 0.23
Urate oxidase	2.33 ± 0.82
FAOS	1194 ± 229
CAT	911 ± 101
CPT	2032 ± 157
FADH	436 ± 44

Values are means ± SD, N = 5.

between control and drug-treated groups, except when PAA, o-CPA, 2,4-D and 2,4,5-T were used (73, 76, 73 and 28% of the control respectively) (data not shown). Hepatomegaly was observed in treatment with PAA (1.18-fold) and 2,4,5-T (1.21-fold) of the first group, all drugs (1.13- to 1.45-fold) of the second group, and all of the perfluorinated compounds (1.13- to 1.45-fold) except for PFBA. The extent of the increase in hepatic weight after treatment with 2,4,5-TP and PFOA exceeded that found in CPIB treatment. No significant change in hepatic protein content was observed after treatment with any of the drugs. The activity of catalase was decreased by statistically significant levels by all of the phenoxyacetic acid derivatives classified into the first group (Table 2) and also by MCPA (Table 3), whereas 2,4-DP, 2,4,5-TP and CPIB significantly induced catalase activity (34, 68 and 44% respectively). Perfluorinated compounds showed no significant effect on catalase activity (Table 4). A decrease in the activity of DAAO was observed only in the

Table 2. Effects of phenoxyacetic acid derivatives on some biochemical values and activities of peroxisomal and mitochondrial enzymes of rat liver

	Relative values (% of control)				
	PAA	o-CPA	p-CPA	2,4-D	2,4,5-T
Liver weight	1.18*	1.17	1.02	1.04	1.21*
Protein content	0.85	0.97	0.94	0.92	0.90
Enzyme: Catalase	0.71*	0.74*	0.66*	0.68*	0.80*
DAAO	1.08	1.10	0.92	1.08	0.92
U. oxid.	0.97	0.88	0.92	0.94	0.88
FAOS	1.06	0.82	1.04	1.69*	2.46*
CAT	0.99	0.82	1.23*	2.22*	8.07*
CPT	1.07	1.01	1.10	1.49*	2.67*
FADH	0.81	1.04	1.09	1.22*	1.61*

Data are presented as the mean values relative to control, N = 5. See Table 1 for control values.
* $P < 0.05$ vs control.

Table 3. Effects of phenoxypropionic acid derivatives on some biochemical values and activities of peroxisomal and mitochondrial enzymes of rat liver

	Relative values (% of control)				
	p-CPP	2,4-DP	2,4,5-TP	MCPA	CPIB
Liver weight	1.13*	1.22*	1.45*	1.13*	1.24*
Protein content	0.98	1.06	1.07	0.87	1.01
Enzyme: Catalase	1.06	1.34*	1.68*	0.74*	1.44*
DAAO	0.76	0.76	0.68*	1.13	0.48*
U. oxid.	0.84	0.69*	0.80	0.96	0.71*
FAOS	2.37*	6.63*	12.47*	2.58	4.54*
CAT	4.40*	50.94*	83.20*	2.59	39.60*
CPT	1.50*	2.61*	1.82*	1.62	2.29*
FADH	1.27	1.75*	1.64*	1.18	1.65*

Data are presented as the mean values relative to control, N = 5. See Table 1 for control values.
* $P < 0.05$ vs control.

Table 4. Effects of perfluorinated compounds on some biochemical values and activities of peroxisomal and mitochondrial enzymes of rat liver

	Relative value (% of control)					
	Control	PFBA	PFOA	PFDA	PFOL	PFOS
Liver weight (% of body wt)	4.10 ± 0.35	1.15	1.45*	1.16*	1.13*	1.16*
Protein content (mg/g liver)	171 ± 21	1.01	0.86	0.83	1.01	0.96
Enzyme (units/g liver):						
Catalase	32.4 ± 6.9	1.10	0.84	1.01	0.82	1.13
DAAO	0.88 ± 0.44	0.98	0.52	0.95	1.57	1.13
U. oxid.	1.86 ± 0.44	1.01	0.83	0.90	1.12	0.75
FAOS	684 ± 155	2.01*	4.33*	3.11*	2.02*	1.14
CAT	522 ± 58	6.75*	17.69*	16.40*	5.89*	2.64*
CPT	2208 ± 268	1.40	1.55*	1.41*	1.40*	1.00
FADH	1066 ± 156	1.37*	1.33*	1.21	1.38*	1.27*

Control values are means ± SD, N = 5. Other data are presented as the mean values relative to control, N = 5.
* $P < 0.05$ vs control.

cases of 2,4,5-TP and CPIB treatment (32 and 52% respectively). A decrease in the activity of urate oxidase was also observed only in the cases of 2,4-DP and CPIB. Concerning fatty acid metabolism-related enzymes, 2,4-D and 2,4,5-T which had two and three chlor-substitutions on the phenyl moiety, respectively, induced significantly the activities of FAOS, CAT, CPT and FADH, and the extent of the induction by 2,4,5-T exceeded that by 2,4-D. However, p-CPA having one chlor-substitution on the phenyl moiety showed an increase in CAT only (Table 2). The effects of drugs classified in the second group, which had one or two methyl substitutions in the alpha position, on those enzymes are shown in Table 3. The activities of FAOS, CAT and CPT were increased significantly by all of the drugs of this group, whereas that of FADH was increased by drugs other than p-CPP. Of those drugs, the effects of 2,4-DP and 2,4,5-TP on the activities of FAOS and CAT were marked, and the potencies of the drugs as inducers for these enzymes were stronger than observed with CPIB. The effects of perfluorinated compounds on these enzymes are shown in Table 4. PFBA, PFOA, PFDA and PFOL induced the activity of FAOS by 2.01-, 4.33-, 3.11- and 2.02-fold respectively. Furthermore, these compounds also induced markedly the activity of CAT. However, the effects of these compounds on the activities of mitochondrial enzymes, FADH and CPT, were less than 1.6-fold. Interestingly, PFOS and PFOL, which represented the sulfonic acid form and the hydroxylmethyl form, also induced some fatty acid metabolism-related enzymes in peroxisomes and mitochondria, although the inducing level was less than that brought about by the corresponding carboxylic form.

The partition of phenoxyacetic acid derivatives into water and octanol was measured, and the correlations among lipophilicity, the number of chlor-substitutions on the phenyl group, the number of methyl groups on the acetic acid moiety and inducibility of peroxisomal FAOS and CAT were examined (Figs. 2 and 3). Octanol–water partition coefficients of the drugs are summarized in Table 5. As the numbers of chlor-substitutions and methyl groups increased, partition coefficients (log P), which are a measure of lipophilicity, also increased. Concerning p-CPA, 2,4-D and 2,4,5-T, the correlation coefficient between log P and the FAOS activity was 0.96 (Fig. 2A), and that between log P and the CAT activity was 0.88. Concerning p-CPP, 2,4-DP and 2,4,5-TP, the correlation coefficients between log P and the FAOS activity and between log P and the CAT activity were 0.92 and 0.96, respectively (Fig. 2B), suggesting high correlation among the number of chlor-substitutions, lipophilicity and inducibility to FAOS and CAT. Furthermore, as shown in Fig. 3, there was a high correlation among the number of methyl groups at the alpha position of the acetic acid moiety, an increase in the partition coefficient and the inducibility of the FAOS and CAT activities.

DISCUSSION

Induction of hepatic peroxisomal fatty acid oxidation by hypolipidemic peroxisome proliferators such as clofibrate and diethyl-hexylphthalate may be due to an enhancement of the rate of transcription and/or increase in the amount of mRNA for the enzyme [21]. However, the relationship between the structures of the drugs and their ability to induce peroxisomal enzymes has not yet been elucidated. To clarify what type of structure may be responsible for the induction of these enzymes, we examined the *in vivo* effect of phenoxyacetic acid derivatives, which are structurally parental compounds of clofibrate (CPIB), on the activities of fatty acid metabolism-related enzymes in hepatic peroxisomes and mitochondria. On the other hand, an induction of peroxisomal fatty acid oxidation has also been found in rats receiving high fat diets [22, 23], suggesting a possible mechanism of induction, i.e. substrate-induction by fatty acid. In the present

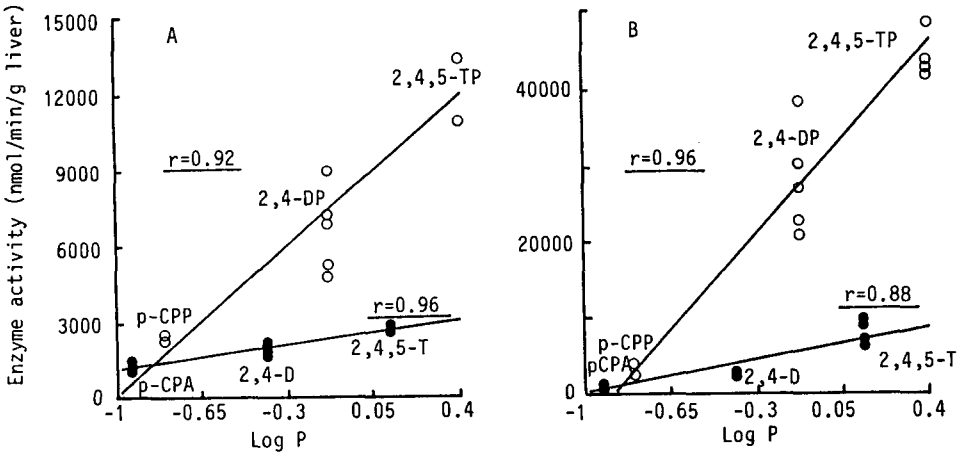

Fig. 2. Correlations between the activities of FAOS (A) and CAT (B) and partition coefficients of phenoxyacetate-derivatives. Each point corresponds to the value from an animal in the treated group, N = 5.

Table 5. Octanol–water partition coefficients (log P) of phenoxyacetic and phenoxypropionic acid derivatives

Compounds	Log P
PAA	−1.78
o-CPA	−1.18
p-CPA	−0.92
2,4-D	−0.32
2,4,5-T	0.13
p-CPP	−0.79
2,4-DP	−0.13
2,4,5-TP	0.39
MCPA	−0.35
CPIB	−0.37

$$P: \frac{\text{Concentration in octanol phase}}{\text{Concentration in water phase}}$$

experiments, we also studied perfluoro-compounds. These compounds have structures quite similar to those of fatty acids, with hydrogen molecules on the alkyl-chain being replaced by fluorine molecules, causing them to be much less susceptible to fatty acid oxidation [24].

Of phenoxyacetic acid derivatives, the derivatives (PAA and CPAs) having less than one chlor-substitution on the phenyl moiety showed no inducing effect on the activities of any enzymes examined. This may be due to failure to accumulate these compounds in the body as a result of their poor lipophilicities (Table 5). On the other hand, although p-CPP, having a methyl group on the alpha position of the acetic acid moiety of CPA, was also poorly lipophilic, increases in some enzyme activities were observed. These results show that in addition to high lipophilicity the methyl-group at the alpha position of the acetic acid moiety may have an important role

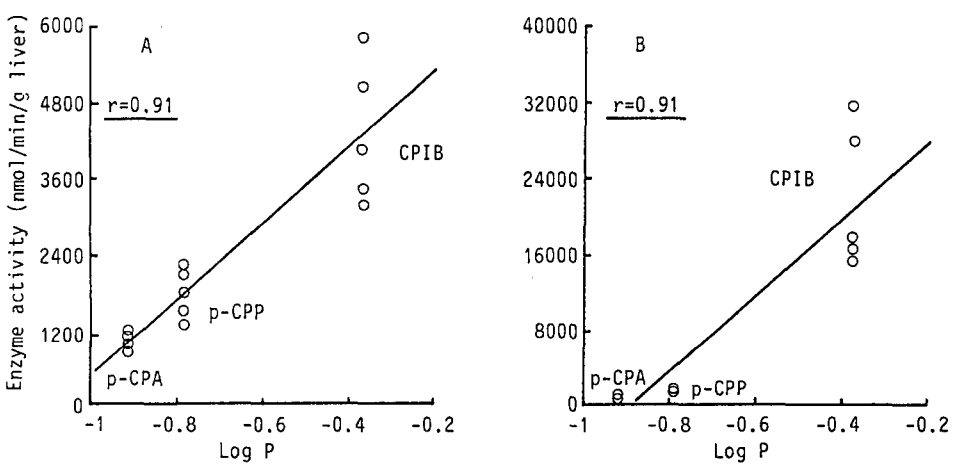

Fig. 3. Correlations between the activities of FAOS (A) and CAT (B) and partition coefficients of p-CPA, p-CPP and CPIB. Each point corresponds to the value from an animal in the treated group, N = 5.

in the induction of peroxisomal and mitochondrial enzymes by clofibrate-related compounds. There is a possibility that drugs incorporated into the cell may be converted to their CoA-forms and further metabolized. The methyl group introduced at the alpha position may cause a steric hindrance for an acylation reaction with CoA-SH catalyzed by acyl-CoA synthetase, resulting in an accumulation of the drugs in liver cells, and thus accumulation of the original forms of the drugs may relate to the induction of hepatic enzymes. Lipophilicity of drugs also seems to be an important factor for enzyme induction. As lipophilicity of a drug increases, the drug tends to accumulate in the body. As the numbers of chlor-substitutions and methyl groups of the drug increase, partition of the drug into octanol phase increases. In the present experiment, there was a high correlation between the activities of FAOS and CAT and lipophilicity of the drugs examined. The order of perfluorinated compounds in the ability to induce hepatic peroxisomal and mitochondrial enzymes was PFOA = PFDA > PFBA. On the other hand, hydroxymethyl- or sulfonic acid derivatives of perfluorooctanoic acid could also induce hepatic FAOS and CAT. Although it has been suggested that the hydroxymethyl form may be metabolized to its carboxylic acid form in the body, and then induce peroxisomal enzyme activities [25], our present data clearly show that hepatic fatty acid oxidation-related enzymes may be induced not only by the carboxylic acid form but also by the sulfonic acid form. Until now, there have been no reports concerning the formation of the CoA-ester of sulfonic acid compounds. From these observations we would like to propose that the formation of CoA-ester of the drug is not essential for the induction process. Thus, we have concluded that the characteristics of chemical structure necessary for induction of hepatic fatty acid oxidation-related enzymes are: (1) similarity to fatty acid, (2) poor susceptibility to fatty acid oxidation, and (3) high lipophilicity and a partition coefficient (log P) in the octanol/water system of more than −1. Recently, Reddy and coworkers [26] reported the presence of a binding protein for the peroxisome proliferator in rat liver. Although the concept of a receptor or binding protein for peroxisome proliferators has not yet been accepted widely, as the number of chlor-substitutions and/or methyl-groups adjacent to the carboxyl group increase, the affinity of the drug to such a binding protein may be increased. Concerning hepatic responses to peroxisome proliferators including induction of peroxisomal enzyme activities, marked species and organ dependency have been found [27]. These facts may support the participation of some binding protein or receptor in the pleiotropic response of the liver to peroxisome proliferators. On the other hand, similar morphological and biological changes in the liver were induced by drugs structurally unrelated to each other, suggesting that there may be a common mechanism for peroxisome proliferation in addition to the three factors described above. Recently, we reported for the first time that calcium antagonists can suppress peroxisome proliferation in rat liver [28] and have suggested that a calcium-dependent mechanism may be responsible for peroxisome proliferation by drugs. To clarify the detailed mechanism of induction of subcellular organelles including peroxisomes, mitochondria and microsomes, we propose to examine a change in the signal transduction system after drug treatment in the near future.

REFERENCES

1. Hess R, Staubli W and Reiss W, Nature of the hepatomegalic effect produced by ethylchlorophenoxyisobutyrate in the rat. *Nature* **208**: 856–858, 1965.
2. Svoboda DJ and Azarnoff AL, Response of hepatic microbodies to a hypolipidemic agent ethylchlorophenoxyisobutyrate (CPIB). *J Cell Biol* **30**: 442–450, 1966.
3. Lalwani ND, Reddy MK, Qureshi SA, Sirtori CR, Abiko Y and Reddy JK, Evaluation of selected hypolipidemic agents for the induction of peroxisomal enzymes and peroxisome proliferation in the rat liver. *Hum Toxicol* **2**: 27–48, 1983.
4. Reddy JK, Svoboda DJ and Azarnoff DL, Microbody proliferation in liver induced by nafenopin, a new hypolipidemic drug. *Biochem Biophys Res Commun* **52**: 537–543, 1973.
5. Lalwani ND, Reddy MK, Qureshi SA and Reddy JK, Development of hepatocellular carcinomas and increased peroxisomal fatty acid beta-oxidation in rats fed Wy 14,643 in the semipurified diet. *Carcinogenesis* **7**: 645–650, 1981.
6. Reddy JK and Krishnakantha TP, Hepatic peroxisome proliferation: Induction by two novel compounds structurally unrelated to clofibrate. *Science* **190**: 787–789, 1975.
7. Barnard SD, Molello JA, Caldwell WJ and LeBeau JE, Comparative ultra-structural study of rat hepatocytes after treatment with the hypolipidemic agents probucol, clofibrate and fenofibrate. *J Toxicol Environ Health* **6**: 547–557, 1980.
8. Moody DE and Reddy JK, The hepatic effects of hypolipidemic drugs (clofibrate, nafenopin, tibric acid and Wy 14,643). *Am J Pathol* **90**: 435–450, 1978.
9. Reddy JK, Rao MS and Moody DF, Hepatocellular carcinomas in acatalasemic mice treated with nafenopin, a hypolipidemic peroxisome proliferator. *Cancer Res* **36**: 1211–1217, 1976.
10. Reddy JK and Rao MS, Malignant tumors in rats fed nafenopin, a hepatic peroxisome proliferator. *J Natl Cancer Lett* **59**: 1645–1650, 1977.
11. Svoboda DJ and Azarnof DL, Tumors in rats fed ethylchlorophenoxyisobutyrate, a hypolipidemic drug. *Cancer Res* **39**: 3419–3428, 1979.
12. Reddy JK and Rao MS, Peroxisome proliferators and cancer. *Trends Pharmacol Sci* **7**: 438–443, 1986.
13. Goel SK, Lalwani ND and Reddy JK, Peroxisome proliferation and lipid peroxidation in rat liver. *Cancer Res* **46**: 1324–1330, 1986.
14. Reddy JK and Lalwani ND, Carcinogenesis by hepatic peroxisome proliferators: Evaluation of the risk of hypolipidemic drugs and industrial plasticizers to humans. *CRC Crit Rev Toxicol* **12**: 1–58, 1983.
15. Lazarow PB and de Duve C, A fatty acyl-CoA oxidizing system in rat liver peroxisomes. *Proc Natl Acad Sci USA* **73**: 2043–2046, 1976.
16. Fritz IB and Schults F, Carnitine acetyltransferase II. Inhibition by carnitine analogues and by sulfhydryl reagents. *J Biol Chem* **240**: 2188–2192, 1965.
17. Hryb DJ and Hogg JF, Chain length specificities of peroxisomal and mitochondrial beta-oxidation in rat liver. *Biochem Biophys Res Commun* **87**: 1200–1205, 1979.

18. Hayashi H, Suga T and Niinobe S, Studies on peroxisomes. I. Intraparticulate localization of peroxisomal enzymes in rat liver. *Biochim Biophys Acta* **252**: 58–68, 1971.
19. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ, Protein measurement with the Folin phenol reagent. *J Biol Chem* **193**: 265–275, 1951.
20. Hashimoto Y, Kishi H, Takano J and Yasuoka T, Determination of partition coefficient. In: *Determination of Physicochemical Properties of Chemicals* (in Japanese) (Ed. Hashimoto Y), pp. 23. Sangyo Tosho Publisher, Tokyo, Japan, 1987.
21. Reddy JK, Goel SK, Nemali MR, Carrino JJ, Laffler TG, Reddy MK, Sperbeck SJ, Osumi T, Hashimoto T, Lalwani ND and Rao MS, Transcriptional regulation of peroxisomal fatty acyl-CoA oxidase and enoyl-CoA hydratase/3-hydroxyacyl-CoA dehydrogenase in rat liver by peroxisome proliferators. *Proc Natl Acad Sci USA* **83**: 1747–1751, 1986.
22. Ishii H, Fukumori N, Horie S and Suga T, Effect of fat content in the diet on hepatic peroxisomes of the rat. *Biochim Biophys Acta* **617**: 1–11, 1980.
23. Neat CE, Thomassen MS and Osmundsen H, Induction of peroxisomal beta-oxidation in rat liver by high-fat diet. *Biochem J* **186**: 369–371, 1980.
24. Ophaug RH and Singer L, Metabolic handling of perfluorooctanoic acid in rats. *Proc Soc Exp Biol Med* **163**: 19–23, 1980.
25. Ikeda T, Aiba K, Fukuda K and Tanaka M, The induction of peroxisome proliferation in rat liver by perfluorinated fatty acids, metabolically inert derivatives of fatty acids. *J Biochem (Tokyo)* **98**: 475–482, 1985.
26. Lalwani ND, Alvares K, Reddy MK, Parikh I and Reddy JK, Peroxisome proliferator-binding protein: Identification and partial characterization of nafenopin-, clofibric acid-, and ciprofibrate-binding proteins. *Proc Natl Acad Sci USA* **84**: 5242–5246, 1987.
27. Watanabe T, Horie S, Yamada J, Isaji M, Nishigaki T, Naito J and Suga T, Species differences in the effects of bezafibrate, a hypolipidemic agent, on hepatic peroxisome-associated enzymes. *Biochem Pharmacol* **38**: 367–371, 1989.
28. Watanabe T and Suga T, Suppression of clofibrate-induced peroxisome proliferation in rat liver by nicardipine, a calcium antagonist. *FEBS Lett* **232**: 293–297, 1988.