Biochemical Pharmacology, Vol. 33, No. 7, pp. 1081–1085, 1984.
Printed in Great Britain.

0006-2952/84 $3.00 + 0.00

INDUCTION OF RAT HEPATIC LONG-CHAIN ACYL-CoA HYDROLASES BY VARIOUS PEROXISOME PROLIFERATORS

HARUYO KATOH,* SETSUKO NAKAJIMA,* YOICHI KAWASHIMA,*‡ HIROSHI KOZUKA* and MITSURU UCHIYAMA†

*Faculty of Pharmaceutical Sciences, Toyama Medical and Pharmaceutical University, 2630 Sugitani, Toyama 930-01, Japan; †National Institute of Hygienic Sciences, 1-18-1 Kamiyoga, Setagaya, Tokyo 158, Japan

(*Received* 17 *June* 1983; *accepted* 21 *October* 1983)

Abstract—Induction of cytosolic long-chain acyl-CoA hydrolases was investigated in rat liver after administration of various peroxisome proliferators and related compounds. Treatment of rats with di-(2-ethylhexyl)-phthalate, di-(2-ethylhexyl)-adipate or tiadenol induced hydrolases I and II, while acetylsalicylic acid induced only hydrolase II. Among the various phenoxyacetic acid derivatives and related compounds, 2,4,5-trichlorophenoxyacetic acid, 2-(4-chlorophenoxy)-2-methylacetic acid, 2-(2-chlorophenoxy)-2-methylpropionic acid and clofibric acid induced both hydrolases I and II, whereas 2, 4-dichlorophenoxyacetic acid induced only hydrolase II. All nine of the above-mentioned inducers also markedly increased the activity of peroxisomal β-oxidation. Other compounds tested (2-chlorophenoxyacetic acid, 4-chlorophenoxyacetic acid, 4-chlorophenol, phenoxyacetic acid and phenoxy-2-methylacetic acid) were ineffective as inducers. These results suggest that inducers of acyl-CoA hydrolase II also enhance peroxisomal β-oxidation activity, but do not necessarily induce acyl-CoA hydrolase I. The structure-inducing activity relationships of these compounds are discussed.

Long-chain acyl-CoA hydrolase is a ubiquitous enzyme and is localized mainly in the microsomes and mitochondria of rat liver [1, 2]. We previously found [3] that two long-chain acyl-CoA hydrolases are induced in the hepatic cytosol of rats following the administration of clofibric acid§; these are acyl-CoA hydrolases I and II, having molecular weights of approximately 80,000 and approximately 40,000, respectively. The inductions of these two long-chain acyl-CoA hydrolases respond differently to changes of hormonal conditions in rats [4]. As with other acyl-CoA hydrolases, the precise physiological role of these hydrolases remains uncertain and the mechanism of the induction also remains unclear. Clofibrate, the ethyl ester of clofibric acid, is used clinically as a hypolipidemic drug and is known to be a potent peroxisome proliferator [5, 6]. Several compounds, including some structurally unrelated to clofibrate, have also been shown to cause peroxisome proliferation [7–10]. DEHP is one such peroxisome proliferator [11], and has been shown to induce a long-chain acyl-CoA hydrolase in the hepatic cytosol of the rat [12]. Taken together with our findings [1], these facts suggest that peroxisome proliferators might generally induce acyl-CoA hydrolases.

DEHP, acetylsalicylic acid and tiadenol are known to induce peroxisomal β-oxidation to different extents [8, 10, 13]. Furthermore, we recently found [14] that 2,4-D and 2,4,5-T (herbicides structurally related to clofibric acid) also induce certain peroxisomal enzymes. Thus, in the present work we investigated the relationship between the inductions of acyl-CoA hydrolases and peroxisomal enzymes in rats by the above-mentioned compounds and other related compounds. It was found that inducers of acyl-CoA hydrolase II also enhanced peroxisomal β-oxidation, but did not necessarily induce acyl-CoA hydrolase I as well.

MATERIALS AND METHODS

Chemicals. Palmitoyl-CoA, myristoyl-CoA, 2-(4-chlorophenoxy)-2-methylacetic acid and 2-(2-chlorophenoxy)-2-methylpropionic acid were purchased from Sigma Chemical Co. (St. Louis, MO). DEHP, DEHA, 2,4-D, 2,4,5-T, 4-chlorophenoxyacetic acid, 2-chlorophenoxyacetic acid, phenoxy-2-methylacetic acid and phenoxyacetic acid were obtained from Tokyo Chemical Industry (Tokyo, Japan); tiadenol was from Aldrich Chemical Co. (Tokyo, Japan). All other chemicals were of analytical grade.

Animals. Male rats of the Wistar strain, weighing 140–180 g, were used. Rats were fed *ad lib.* on a regular commercial diet (obtained from Sankyo Labo Service Co., Tokyo, Japan) or a diet containing one of the agents for 1 or 2 weeks. The concentrations of test compounds in the diet were 2% for DEHP and DEHA, 0.5% for tiadenol, 1.0% for acetylsalicylic acid, 0.25% for 2,4-D and 2,4,5-T, and 0.5% for other phenoxyacetic acid derivatives. Phenoxyacetic acid derivatives were also administered to rats sub-

‡ To whom correspondence should be addressed.

§ Abbreviations: clofibric acid, 2-(4-chlorophenoxy)-2-methylpropionic acid; 2,4-D, 2,4-dichlorophenoxyacetic acid; 2,4,5-T, 2,4,5-trichlorophenoxyacetic acid; DEHP, di-(2-ethylhexyl)-phthalate; DEHA, di-(2-ethylhexyl)-adipate.

Table 1. Induction of acyl-CoA hydrolases and peroxisomal β-oxidation by DEHP, DEHA, acetylsalicylic acid and tiadenol

Treatment	Hydrolase I	Hydrolase II	Palmitoyl-CoA oxidation	Catalase
	Units/g liver			
Control	0	0	0.57 ± 0.1	34.7 ± 4.72
2% DEHP	2.29	1.04	5.74 ± 0.6	50.7 ± 10.8
	1.54	0.99		
2% DEHA	0.51	0.76	2.39 ± 0.5	52.1 ± 5.2
	0.38	0.57		
1% Acetylsalicylic acid	0	0.42	2.76 ± 0.6	45.7 ± 7.1
	0	0.48		
0.5% Tiadenol	5.65	1.16	7.04 ± 1.2	75.0 ± 8.9
	4.73	0.95		

Cytosol was prepared from at least two pooled livers, and 50 mg cytosolic protein was subjected to chromatography on a Sephadex G-100 column (2.2 × 85 cm) equilibrated with 0.1 M potassium phosphate buffer (pH 7.4). The enzyme activity of each fraction was assayed spectrophotometrically with palmitoyl-CoA as the substrate, and the activities corresponding to each peak of hydrolase were integrated. Agents were administered to rats in the diet for 7 days at the dose described in the text. The results are given as means ± S.D. from three or four separate experiments. A value of zero means below the limit of detection (2×10^{-3} unit/ml of each fraction).

cutaneously. Each compound was dissolved in 0.9% NaCl as the sodium salt at a concentration of 1.4 mmole/ml, and the solution was injected into the rats once a day for 7 days at a dose of 0.93 mmole or 1.86 mmole per kg of body weight.

Cytosol and post-nuclear supernatant were obtained as described previously [15].

Enzyme assays. Long-chain acyl-CoA hydrolase was assayed spectrophotometrically using palmitoyl-CoA or myristoyl-CoA as the substrate after gel filtration of the cytosol through a Sephadex G-100 column as described previously [3, 15]. One unit of acyl-CoA hydrolase is defined as the activity required to hydrolyze 1 μmole of acyl-CoA per min at 30°. Peroxisomal β-oxidation was assayed by the method of Lazarow and de Duve [16] with minor modifications as described previously [4]. Catalase activity was determined by the method of Aebi [17]. Post-nuclear supernatant was used as the enzyme source for peroxisomal β-oxidation and catalase assay.

Protein concentrations in the enzyme preparations were determined by the method of Lowry *et al.* [18] using bovine serum albumin as the standard.

RESULTS

Table 1 shows the effects of the administration of DEHP, DEHA, acetylsalicylic acid and tiadenol, which have been reported to be peroxisome proliferators [8–11], on the induction of hepatic hydrolases I and II and on the activities of peroxisomal β-oxidation and catalase. Tiadenol was the most potent inducer of hydrolase I, peroxisomal β-oxidation and catalase, being more potent than clofibric acid as an inducer of the former two activities (Tables 1 and 2). Tiadenol was also reported to increase the activity of palmitoyl-CoA hydrolase in the cytosolic fraction of rat liver [10]. A long-chain acyl-CoA hydrolase has been purified from the hepatic cytosol of rats treated with DEHP [12]. Out present study confirmed that the increase in activity in the cytosolic fraction is due to the induction of two long-chain acyl-CoA hydrolases, as is the case with clofibric acid treatment. Acetylsalicylic acid increased peroxisomal β-oxidation and catalase activities to about the same extent as DEHA did, but hydrolase II was induced less effectively than by DEHA, and hydrolase I was not induced at all. Administration of DEHP increased peroxisomal β-oxidation 10 times over the control level and induced hydrolase II to the same extent as did tiadenol and clofibric acid, whereas the induction of hydrolase I was less marked. Catalase activity was increased by all four agents, although the increase was, at most, twice the control level.

Table 2 shows the effects of various phenoxyacetic acid derivatives and related compounds. Administration at 0.5% in the diet meant that rats received approximately three times greater amounts of compounds than those injected at the lower doses. 2,4-D and 2,4,5-T were administered at a dietary concentration of 0.25% owing to their toxic effect on rats at 0.5%. Hydrolase I was induced by dietary administration of 2-(4-chlorophenoxy)-2-methylacetic acid, although the extent of the induction was somewhat less than that by clofibric acid; however, hydrolase II was induced to similar extents by both compounds. Neither hydrolase I nor hydrolase II was induced by the dietary administration of 4-chlorophenoxyacetic acid or 2-chlorophenoxyacetic acid. Similar results were obtained when the compounds were administered subcutaneously (experiment II).

In the subcutaneous administration experiments, clofibric acid, 2-(4-chlorophenoxy)-2-methylacetic acid and 2-(2-chlorophenoxy)-2-methylpropionic acid induced hydrolase II at both doses, whereas hydrolase I was not induced by 2-(4-chlorophenoxy)-2-methylacetic acid or by 2-(2-chlorophenoxy)-2-methylpropionic acid at the lower dose. Other compounds tested subcutaneously had no inducing effects.

On the administration of 2,4,5-T for 1 or 2 weeks

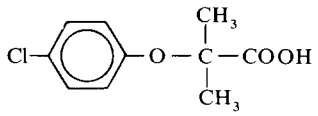
Table 2. Induction of acyl-CoA hydrolases and peroxisomal enzymes in rat liver by treatment with phenoxyacetic acid derivatives and related compounds

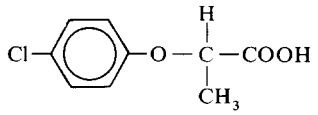
Treatment	Dose mmoles/kg	Hydrolase I	Hydrolase II	Catalase	Palmitoyl-CoA oxidation
		Units/g liver			
Experiment I (diet)					
Control		0	0	32.0 ± 8.2	0.28 ± 0.07
Clofibric acid		3.28	1.81	76.1 ± 14.5	3.95 ± 0.54
		1.72	1.15		
2-(4-Chlorophenoxy)-2-methyl-acetic acid		2.09	1.12	59.7 ± 5.8	4.84 ± 0.16
		1.80	1.44		
4-Chlorophenoxyacetic acid		0	0	25.4 ± 3.3	0.44 ± 0.90
		0	0		
2-Chlorophenoxyacetic acid		0	0	29.8 ± 4.2	0.81 ± 0.10
		0	0		
Experiment II (s.c.)					
Clofibric acid	0.93	3.99	1.17	35.6 ± 10.0	4.74 ± 1.22
	1.86	1.44	0.87	n.d.	n.d.
		1.94	0.73		
2-(4-Chlorophenoxy)-2-methyl-acetic acid	0.93	0	0.33	35.6 ± 6.2	1.94 ± 0.43
	1.86	0.44	1.14	37.3 ± 10.2	2.36 ± 0.64
		0	0.79		
2-(2-Chlorophenoxy)-2-methyl-propionic acid	0.93	0	0.45	28.0	1.42
				36.1	2.03
	1.86	1.23	0.72	42.8	4.67
				57.8	4.54
4-Chlorophenoxyacetic acid	0.93	0	0	n.d.	n.d.
	1.86	n.d.	n.d.	34.3	0.55
2-Chlorophenoxyacetic acid	0.93	0	0	n.d.	n.d.
4-Chlorophenol	1.86	0	0	33.0 ± 5.74	0.37 ± 0.08
Phenoxyacetic acid	0.93	0	0	n.d.	n.d.
Phenoxy-2-methylacetic acid	0.93	0	0	n.d.	n.d.
	1.86	0	0	34.0 ± 10.3	1.01 ± 0.24
Experiment III (diet)					
2,4-D 7 days		0	0.66	41.4 ± 5.6	2.29 ± 0.43
		0	0.50		
14 days		0	0.38	n.d.	n.d.
		0	0.30		
2,4,5-T 7 days		0.61	1.30	40.7 ± 9.7	3.92 ± 0.10
		0.60	1.29		
14 days		0.74	1.07	n.d.	n.d.
		0.89	1.18		

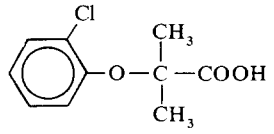
Cytosol was prepared from at least two pooled livers. The activities of hydrolases I and II were estimated with myristoyl-CoA as the substrate after gel filtration of the cytosol in the same manner as described in Table 1. In experiment I, rats were fed a diet containing one of the test compounds at a dose of 0.5% for 7 days. In experiment II, rats were injected with the compounds subcutaneously for 7 days. In experiment III, rats were fed a diet containing 2,4-D or 2,4,5-T at a dose of 0.25% for 7 or 14 days. One unit of palmitoyl-CoA oxidation is defined as the activity required to reduce 1 μmole of NAD per min at 37°. Where shown, values are means ± S.D. from three to eight separate experiments. n.d., Not determined. A value of zero means below the limit of detection (2×10^{-3} unit/ml of each fraction).

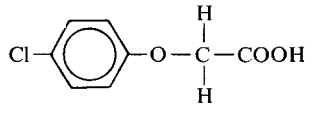
to rats in the diet (experiment III), hydrolase I was induced in the cytosol (approximately one-third of that induced by clofibric acid). On the other hand, treatment with 2,4-D did not induce any activity of hydrolase I under the present experimental conditions. Hydrolase II was induced by both compounds. Although hydrolase II induction by 2,4,5-T was similar to that by clofibric acid (that by 2,4-D was less), the induction by clofibric acid was significantly greater on the basis of whole liver, owing to the difference in hepatomegalic effects of these compounds (data not shown).

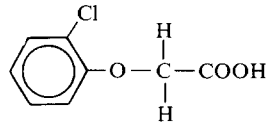
The inducing effects of the test compounds on peroxisomal enzymes were also determined for comparison with the patterns of induction of the hydrolases. Feeding of rats with clofibric acid, 2-(4-chlorophenoxy)-2-methylacetic acid or 2,4,5-T increased the activity of cyanide-insensitive palmitoyl-CoA oxidation approximately 15 times over the control. Treatment with 2,4-D also increased the activity, whereas 4-chlorophenoxyacetic acid or 2-chlorophenoxyacetic acid had little effect. The activity of catalase was increased by clofibric acid or 2-(4-chlorophenoxy)-2-methylacetic acid treatment, but only to approximately twice the control level. Peroxisomal β-oxidation activity was also increased effectively by the subcutaneous administration of clofibric acid or 2-(4-chlorophenoxy)-2-methylacetic acid, as in the feeding experiments. 2-(2-Chlorophenoxy)-2-methylpropionic acid and 2-phenoxypropionic acid were also effective (experiment II). The activity of catalase was not greatly increased by any of the compounds tested under the present experimental conditions. It should be noted that hepatomegaly

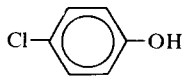

Clofibric acid

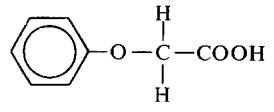

2-(4-Chlorophenoxy)-2-methylacetic acid

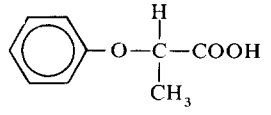

2-(2-Chlorophenoxy)-2-methylpropionic acid

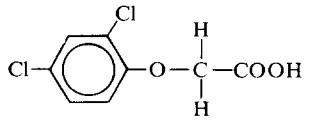

4-Chlorophenoxyacetic acid


2-Chlorophenoxyacetic acid


4-Chlorophenol


Phenoxyacetic acid


Phenoxy-2-methylacetic acid


2,4-Dichlorophenoxyacetic acid (2,4-D)

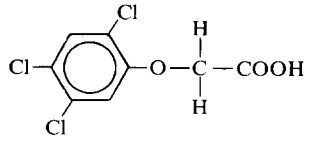

2,4,5-Trichlorophenoxyacetic acid (2,4,5-T)

Fig. 1. Structures of phenoxyacetic acid derivatives and related compounds.

was produced by administration of clofibric acid, 2-(4-chlorophenoxy)-2-methylacetic acid or 2,4,5-T (data not shown).

DISCUSSION

Our recent study on species difference in the induction of hepatic cytosolic acyl-CoA hydrolase by clofibric acid [19] indicated that hydrolases I and II were induced in rats, but only hydrolase II in mice; hepatic peroxisomal β-oxidation was induced in both species. However, neither hydrolase nor peroxisomal β-oxidation was induced in the guinea-pig. From these observations on species difference, it seemed likely that hydrolase II, but not necessarily hydrolase I, is induced when peroxisomal β-oxidation is induced. On the other hand, it was found that hydrolases I and II and peroxisomal β-oxidation all responded differently to hormonal changes in rats [4]. These intriguing observations raised the question of whether there is a definite relationship between the induction of peroxisomal β-oxidation and that of acyl-CoA hydrolase II. In the present study, therefore, we first investigated the induction in rats of hepatic cytosolic acyl-CoA hydrolases by DEHP, DEHA, tiadenol and acetylsalicylic acid, which have been shown by other investigators to induce peroxisomal β-oxidation. The variations in the induction of hydrolase II by the four agents appeared to be similar to those of peroxisomal β-oxidation. We next administered various phenoxyacetic acid derivatives and related compounds to rats in order to investigate the effect of structural variation on the inducing activities. It is evident from Table 2 that compounds which induce hydrolase II in rats also induce peroxisomal β-oxidation, but do not necessarily induce hydrolase I. Therefore, our present results taken together with our previous findings on species difference suggest that the induction of hydrolase II may generally occur when peroxisomal β-oxidation is enhanced, whereas more specific structural features in the inducer may be necessary for the induction of hydrolase I.

The induction of hydrolase I by clofibric acid shows a sex-related difference [15], so the possibility of involvement of metabolite(s) generated by the testosterone-dependent drug metabolizing system in male rat liver [20] must be considered. However, it has been reported that clofibric acid itself is the pharmacologically active principle, and that almost all clofibric acid administered is excreted in the form of the free compound or its glucuronate ester [21]. The present results suggest that phenoxyacetic acid derivatives having one Cl on the phenyl group and more than one methyl group at C-2 of the side chain, or derivatives having more than one Cl on the phenyl group are able to induce both hydrolases or hydrolase II alone. It is particularly noteworthy that among the phenoxyacetic acid derivatives, 2,4-D and 2,4,5-T, which have been used as herbicides, induce one or both of hydrolases I and II. This may be significant in relation to human health, even though the precise physiological role of the induced hydrolases remains to be elucidated. Further work on the mechanism of induction of the hydrolases is in progress in our laboratory.

Acknowledgements—This work was supported in part by grant No. 58771643 for the research of sciences from the Department of Education, Sciences and Culture, Japan.

REFERENCES

1. R. K. Berge, *Biochim. biophys. Acta* **574**, 321 (1979).
2. R. K. Berge and M. Farstad, *Eur. J. Biochem.* **96**, 393 (1979).
3. Y. Kawashima, H. Katoh and H. Kozuka, *J. pharm. Dynam.* **4**, 711 (1981).
4. Y. Kawashima, H. Katoh and H. Kozuka, *Biochim. biophys. Acta* **750**, 365 (1983).
5. R. Hess, W. Stäubli and W. Riess, *Nature, Lond.* **208**, 856 (1965).
6. D. J. Svoboda and D. L. Azarnoff, *J. Cell Biol.* **30**, 442 (1966).
7. J. K. Reddy and T. P. Krishnakantha, *Science* **190**, 787 (1975).
8. T. Sakurai, S. Miyazawa, T. Osumi, S. Furuta and T. Hashimoto, *Toxic. appl. Pharmac.* **59**, 8 (1981).
9. D. E. Moody and J. K. Reddy, *Toxic. appl. Pharmac.* **45**, 497 (1978).
10. R. K. Berge and O. M. Bakke, *Biochem. Pharmac.* **30**, 2251 (1981).
11. J. K. Reddy, D. E. Moody, D. L. Azarnoff and M. S. Rao, *Life Sci.* **18**, 941 (1976).
12. S. Miyazawa, S. Furuta and T. Hashimoto, *Eur. J. Biochem.* **117**, 425 (1981).
13. T. Osumi and T. Hashimoto, *J. Biochem.* **83**, 1361 (1978).
14. Y. Kawashima, H. Katoh, S. Nakajima, H. Kozuka and M. Uchiyama, *Biochem. Pharmac.* **33**, 241 (1984).
15. Y. Kawashima, H. Katoh and H. Kozuka, *Biochim. biophys. Acta* **712**, 48 (1982).
16. P. B. Lazarow and C. De Duve, *Proc. natn. Acad. Sci. U.S.A.* **73**, 2043 (1976).
17. H. Aebi, in *Methods of Enzymatic Analysis* (Ed. H. U. Bergmeyer), Vol. 2, pp. 673–684. Academic Press, New York (1974).
18. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
19. Y. Kawashima, H. Katoh, S. Nakajima and H. Kozuka, *Biochim. biophys. Acta* **752**, 182 (1983).
20. R. Kato and K. Onoda, *Biochem. Pharmac.* **19**, 1649 (1969).
21. M. N. Cayen, E. S. Ferdinandi, E. Greselin, W. T. Robinson and D. Dvornik, *J. Pharmac. exp. Ther.* **200**, 33 (1976).