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Effects of Tiadenol and Di-(2-ethylhexyl)phthalate on the Metabolism of Phosphatidylcholine and Phosphatidylethanolamine in the Liver of Rats

Comparison with Clofibric Acid

Hiroki Mizuguchi, Naomi Kudo,† Takeshi Ohya† and Yoichi Kawashima†‡*

†Faculty of Pharmaceutical Sciences, Josai University, Saitama 350-0295; and *Research Laboratories, Torii Pharmaceutical Co., Ltd., Chiba 267-0056, Japan

ABSTRACT. Metabolic changes induced by 2,2′-(decamethylenedithio)diethanol (tiadenol) and di-(2-ethylhexyl)phthalate (DEHP) in the biosynthesis of phosphatidylcholine (PtdCho) and phosphatidylethanolamine (PtdEtn) in rat liver were compared with changes induced by *p*-chlorophenoxyisobutyric acid (clofibric acid). Treatment of rats with either tiadenol or DEHP increased the hepatic contents of PtdCho and PtdEtn, as was observed with clofibric acid treatment. The administration of tiadenol, DEHP, or clofibric acid slightly, but significantly, increased, in common, the activity of CTP:phosphocholine cytidylyltransferase, a key enzyme for the synthesis *de novo* of PtdCho, and suppressed the activity of PtdEtn *N*-methyltransferase. With regard to the enzymes involved in the synthesis of PtdEtn, the three peroxisome proliferators enhanced the activity of phosphatidylserine (PtdSer) decarboxylase and markedly decreased the activity of CTP:phosphoethanolamine cytidylyltransferase. Treatment of rats with the three compounds markedly increased, in common, the content and the proportion of the molecular species of PtdCho containing oleic acid (18:1), but considerably decreased the proportion of the molecular species of PtdCho containing linoleic acid (18:2) in the liver, resulting in a striking decrease in the concentration of the molecular species of PtdCho containing 18:2 in the serum. The present study suggests that the administration of peroxisome proliferators to rats increases the contents of hepatic PtdCho and PtdEtn for hepatomegaly and proliferation of organelles by the same mechanism, irrespective of their chemical structures. BIOCHEM PHARMACOL **57**;8:869–876, 1999. © 1999 Elsevier Science Inc.

KEY WORDS. phosphatidylcholine; phosphatidylethanolamine; tiadenol; di-(ethylhexyl)phthalate; clofibric acid; peroxisome proliferator

There are a number of compounds that, despite great dissimilarity in their chemical structures, are capable of inducing peroxisome proliferation in the livers of rodents. These compounds are designated "peroxisome proliferators" and are utilized as hypolipidemic agents [clofibrate (ethyl ester of *p*-chlorophenoxyisobutyric acid), bezafibrate, and tiadenol], plasticizers (DEHP§ and dibutyl phthalate), and herbicides (2,4-dichlorophenoxyacetic acid and 2,4,5-trichlorophenoxyacetic acid). The current noteworthy findings in the field of peroxisome proliferators are demonstrations of peroxisome proliferator-activated receptors and peroxisome proliferator-responsive elements [1–3]. These findings have promoted an understanding of the mechanism of induction by peroxisome proliferators of enzymes, such as peroxisomal acyl-CoA oxidase [4] and cytochrome P450 4A6 [5]. In contrast to enzyme induction, the mechanism of peroxisome proliferation has been poorly understood. It has been known that hepatocytes from rodents respond sensitively to peroxisome proliferators to produce hepatomegaly and proliferation of not only peroxisomes, but also mitochondria and endoplasmic reticulum. To achieve hepatomegaly and proliferation of organelles, hepatocytes must be required to increase the supply of phospholipids and proteins as components of biologic membranes. Compared with the extensive investigation of protein import into peroxisomes [6], little is known about the effects of peroxisome proliferators on the biosynthesis of phospholipids in the liver.

Our previous studies showed that the administration of clofibric acid (*p*-chlorophenoxyisobutyric acid), a typical peroxisome proliferator, to rats increases the hepatic contents of PtdCho and PtdEtn by inducing alterations in the activities of the enzymes that participate in the biosynthesis of PtdCho and PtdEtn [7, 8]. However, these previous findings raised a question of whether the alterations induced by clofibric acid in the biosynthesis of PtdCho and PtdEtn also are induced by peroxisome proliferators whose structures differ from that of clofibric acid. If the metabolic alterations induced by clofibric acid are brought about to

‡ Corresponding author: Yoichi Kawashima, Ph.D., Faculty of Pharmaceutical Sciences, Josai University, Keyakidai, Sakado, Saitama 350-0295, Japan. FAX 81-492-71-7984; E-mail: ykawash@josai.ac.jp

§ *Abbreviations*: DEHP, di-(2-ethylhexyl)phthalate; PtdCho, phosphatidylcholine; PtdEtn, phosphatidylethanolamine; PtdIns, phosphatidylinositol; and PtdSer, phosphatidylserine.

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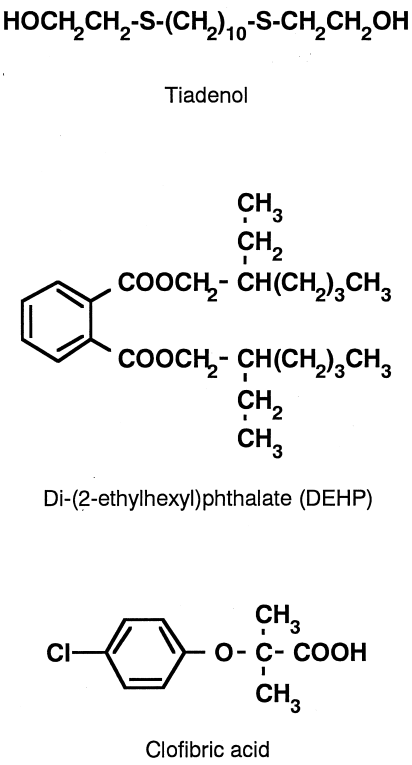

FIG. 1. Chemical structures of tiadenol, DEHP, and clofibric acid.

supply PtdCho and PtdEtn for the proliferation of organelles and for hepatomegaly, do all peroxisome proliferators produce metabolic alterations similar to those induced by clofibric acid? To answer this question, we chose two peroxisome proliferators, tiadenol [2,2′-(decamethylenedithio)diethanol] and DEHP, whose structures are diverse from each other and different from that of clofibric acid (Fig. 1). We studied the responses to these two peroxisome proliferators of hepatic enzymes that are involved in the synthesis of PtdCho and PtdEtn and compared them with the response to clofibric acid.

MATERIALS AND METHODS

Materials

L-3-Phosphatidyl-L-[3-^{14}C]serine (dioleoyl) (55 Ci/mol) and [*methyl*-^{14}C]choline phosphate (55 Ci/mol) were purchased from Amersham. L-[^{14}C-U]Glycerol-3-phosphate (153.2 Ci/mol), [*methyl*-^{14}C]choline (53 Ci/mol), and cytidine diphospho-[*methyl*-^{14}C]choline (55.5 Ci/mol) were from Dupont-New England Nuclear. *S*-Adenosyl-L-[*methyl*-^{14}C]methionine (47 Ci/mol) [1,2-^{14}C]ethanolamine (100Ci/mol) from ICN Biochemicals. Clofibric acid, CDP-choline, CDP-ethanolamine, choline phosphate, ethanolamine phosphate, palmitoyl-CoA, phospholipase C (from *Clostridium welchii*), and BSA were obtained from Sigma; *N*-methyl-PtdEtn, PtdCho (from egg), and PtdSer (from brain) were from Avanti Polar Lipids; *S*-adenosyl-L-methionine was from Boehringer-Mannheim; CTP was from Yamasa Biochemicals; Tween 20 was from Wako Chemicals; and Triton X-100 (reduced) was from Nakalai Tesque. Diacylglycerol and [1,2-^{14}C]ethanolamine phosphate were prepared enzymatically, as described previously [8].

Animals and Preparation of Enzyme Sources

Male Wistar rats, weighing 170–190 g, were obtained from SLC. Rats were fed a standard diet (F-2, Sankyo Labo Service Co.) or a diet that contained 0.15% (w/w) tiadenol, 2% (w/w) DEHP, or 0.5% (w/w) clofibric acid for 7 days, and then were decapitated under light ether anesthesia. Livers and blood were isolated. The livers were perfused with ice-cold 0.9% NaCl and cut into three parts. One of these parts and serum, which was prepared from blood by centrifugation, were stored at −80° until used for the lipid analyses.

The second part of the liver was homogenized in 3 vol. of 0.25 M sucrose, 1 mM EDTA, 10 mM Tris–HCl buffer (pH 7.4). The homogenates were centrifuged at 18,000 *g* for 20 min, and the supernatant was recentrifuged under the same conditions. The resulting supernatant was used as a post-mitochondrial fraction. Microsomes and cytosol were prepared from the post-mitochondrial fraction as described previously [7]. For the preparation of a mitochondrial fraction, the third part of liver was homogenized in 9 vol. of 0.25 M sucrose, 0.1 mM EDTA, 10 mM Tris–HCl buffer (pH 7.4). The homogenates were centrifuged at 600 *g* for 10 min. The supernatant was centrifuged at 5000 *g* for 10 min. The pellet was suspended in the original volume of the homogenizing buffer and recentrifuged under the same conditions. The resulting pellet was washed again in the same manner. The pellet obtained was resuspended in a small volume of 0.25 M sucrose containing 10 mM Tris–HCl (pH 7.4) and was used as a mitochondrial fraction. All operations were carried out at 0–4°.

Enzyme Assays

Glycerol-3-phosphate acyltransferase activity was measured in microsomes by the method of Yamada and Okuyama [9] using [^{14}C]glycerol-3-phosphate and palmitoyl-CoA. The activities of choline kinase and ethanolamine kinase in cytosol were measured using [*methyl*-^{14}C]choline and [^{14}C]ethanolamine according to Ishidate *et al.* [10] with some modifications, as described previously [7, 8]. CTP: phosphocholine cytidylyltransferase in the post-mitochondrial fraction was measured using [*methyl*-^{14}C]choline phosphate in the presence and absence of PtdCho (from egg) and oleic acid (1:1) vesicles according to Ishidate *et al.* [10]. CTP:phosphoethanolamine cytidylyltransferase in the post-mitochondrial fraction was measured according to Sundler [11], using [^{14}C]ethanolamine phosphate with some modifications, as described previously [8]. The activity of CDP-choline:diacylglycerol cholinephosphotransferase was measured in microsomes according to Ishidate *et al.* [10], using CDP-[*methyl*-^{14}C]choline and diacylglycerol (from egg PtdCho) added in a Tween dispersion. The

TABLE 1. Effects of tiadenol, DEHP, and clofibric acid on rat liver

	Control	Tiadenol	DEHP	Clofibric acid
Body weight (g)	194 ± 11	180 ± 9	180 ± 6	190 ± 12
Liver weight (g)	11.2 ± 0.8	16.3 ± 1.0*	14.8 ± 0.6†	15.8 ± 1.9*
Relative liver weight (g/100 g body weight)	5.79 ± 0.13	9.05 ± 0.15*	8.24 ± 0.06*	8.29 ± 0.53*
DNA (mg/g liver)	4.69 ± 0.35	4.60 ± 0.20	4.44 ± 0.25	4.69 ± 0.36
Protein (mg/g liver)	142 ± 2	156 ± 8†	169 ± 5*	155 ± 4†
Phospholipids (μmol/g liver)	29.4 ± 1.3	37.3 ± 0.9*	36.8 ± 2.6*	41.0 ± 0.7*
Peroxisomal β-oxidation (μmol/min/g liver)	0.42 ± 0.11	2.93 ± 0.30*	2.74 ± 0.58*	2.82 ± 0.25*

Rats were fed a control diet or a diet containing 0.15% (w/w) tiadenol, 2% (w/w) DEHP, or 0.5% (w/w) clofibric acid for 7 days. Values are the means ± SD for four rats.
*,†Significantly different from control: *$P < 0.001$, and †$P < 0.05$.

activity of PtdSer decarboxylase in mitochondria was measured using phosphatidyl-[3-^{14}C]serine according to Houweling *et al.* [12]. The activity of PtdEtn *N*-methyltransferase in microsomes was measured according to Audubert and Vance [13] using *S*-adenosyl-L-[^{14}C]methionine in the absence and presence of *N*-methyl-PtdEtn. All assays were confirmed to be proportional to the time and the amounts of protein employed. The substrate concentrations employed gave maximal activities. Peroxisomal β-oxidation was assayed as the activity of cyanide-insensitive palmitoyl-CoA oxidation according to Lazarow and de Duve [14], using homogenates as an enzyme source.

Lipid Analyses

After the addition of tripentadecanoin as an internal standard for the determination of triacylglycerol, lipid was extracted from livers by the method of Bligh and Dyer [15]. Phospholipids were separated by TLC [16], and lipid phosphorus in scrapes of TLC plates was determined according to Rouser *et al.* [17]. Triacylglycerol was separated by TLC on silica gel G, which was developed with *n*-hexane/diethyl ether/acetic acid (80:30:1, by vol.), and triacylglycerol was extracted, methylated, and analyzed by GLC [18]. Analyses of the molecular species of the diacyl form of PtdCho and PtdEtn were carried out according to Blank *et al.* [19] with some modifications, as described previously [20]. Cholesterol in the lipid extract was determined according to Zurkowski [21].

Other Analytical Procedures

Concentrations of protein were measured by the method of Lowry *et al.* [22] with BSA as a standard. DNA in the homogenates was determined according to Kissane and Robins [23].

Statistical Analysis

Statistical significance was determined by one-way ANOVA followed by Schéffe's multiple range test.

RESULTS

Effects of Tiadenol, DEHP, and Clofibric Acid on the Content of Phospholipid in the Liver

Table 1 shows the effects of dietary treatments of rats with 0.15% (w/w) tiadenol, 2% (w/w) DEHP, or 0.5% (w/w) clofibric acid for 7 days on body weight and several hepatic parameters. The protocol for the treatments was chosen to induce peroxisomal β-oxidation by the three different types of peroxisome proliferators to the same extent. Administration of the three peroxisome proliferators to rats caused hepatomegaly and significantly increased the hepatic content of phospholipids to a similar extent, regardless of their chemical structures. No significant change was induced in the hepatic DNA content by the peroxisome proliferators, so that the concentrations of phospholipids in the hepatocytes of the rats treated with the peroxisome proliferators were 1.3- or 1.4-fold those of the control.

An increase in the mass of phospholipids induced in the liver by tiadenol and DEHP was due to the elevated contents of PtdCho, PtdEtn, and PtdIns, as was observed with clofibric acid (Table 2). The content of PtdCho increased 1.3- or 1.4-fold following the administration of the three peroxisome proliferators. The content of PtdEtn was increased 1.8-fold by clofibric acid, whereas tiadenol

TABLE 2. Effects of tiadenol, DEHP, and clofibric acid on the hepatic contents of lipids

	Lipids (μmol/g liver)					
	Phospholipids					
Treatment	PtdCho	PtdEtn	PtdIns	PtdSer	Triacylglycerol	Cholesterol
Control	14.91 ± 0.94	6.91 ± 0.34	2.70 ± 0.27	2.03 ± 0.14	5.18 ± 0.59	4.52 ± 0.44
Tiadenol	20.52 ± 0.70*	8.49 ± 0.26*	3.56 ± 0.29*	1.99 ± 0.09	3.54 ± 0.35*	3.96 ± 0.20
DEHP	19.49 ± 1.63*	9.14 ± 0.44*	3.20 ± 0.29	2.30 ± 0.10	2.63 ± 0.33*	4.53 ± 0.19
Clofibric acid	19.20 ± 0.23*	12.83 ± 0.49*	3.65 ± 0.07*	2.02 ± 0.14	2.52 ± 0.31*	3.67 ± 0.37*

Rats were fed a control diet or a diet containing 0.15% (w/w) tiadenol, 2% (w/w) DEHP, or 0.5% (w/w) clofibric acid for 7 days. Values are the means ± SD for four rats.
*Significantly different from control: $P < 0.001$.

TABLE 3. Alterations by tiadenol, DEHP, and clofibric acid in activities of enzymes that participate in PtdCho and PtdEtn biosynthesis

Enzymes	Control	Tiadenol	DEHP	Clofibric acid
Glycerol-3-phosphate acyltransferase	10.72 ± 2.02	28.52 ± 2.20*	14.59 ± 1.17†	27.78 ± 4.72*
PtdCho synthesis				
Choline kinase	2.46 ± 0.41	1.94 ± 0.27	2.40 ± 0.12	3.10 ± 0.29
CTP:phosphocholine cytidylyltransferase				
Minus lipid vesicles	0.61 ± 0.24	0.32 ± 0.16	0.29 ± 0.16†	0.28 ± 0.10
Plus lipid vesicles	3.30 ± 0.21	3.86 ± 0.23†	4.10 ± 0.42‡	4.36 ± 0.43*
CDP-choline:diacylglycerol cholinephosphotransferase				
Endogenous substrate	0.38 ± 0.03	0.78 ± 0.19‡	0.51 ± 0.12	0.40 ± 0.05
Exogenous substrate	31.37 ± 0.71	29.07 ± 2.39	22.35 ± 3.00‡	27.89 ± 3.08
PtdEtn *N*-methyltransferase				
Endogenous substrate	3.39 ± 0.18	3.95 ± 0.07	3.58 ± 0.28	3.32 ± 0.55
Exogenous substrate	11.35 ± 0.37	9.08 ± 0.34*	8.97 ± 0.79*	9.33 ± 0.49‡
PtdEtn synthesis				
Ethanolamine kinase	1.46 ± 0.02	1.37 ± 0.17	1.52 ± 0.14	1.39 ± 0.11
CTP:phosphoethanolamine cytidylyltransferase	5.20 ± 0.22	3.64 ± 0.25*	2.98 ± 0.30*	3.78 ± 0.53*
PtdSer decarboxylase	0.55 ± 0.02	0.80 ± 0.04*	0.90 ± 0.04*	0.84 ± 0.03*

Rats were fed a control diet or a diet containing 0.15% (w/w) tiadenol, 2% (w/w) DEHP, or 0.5% (w/w) clofibric acid for 7 days. Values, expressed in nmol/min/mg protein, are the means ± SD for four or six rats.

*–‡Significantly different from control: *$P < 0.001$; ‡$P < 0.01$; and †$P < 0.05$.

and DEHP elevated the hepatic content of PtdEtn by 1.2- and 1.3-fold, respectively. In contrast to the phospholipids, the hepatic level of triacylglycerol was decreased by the three peroxisome proliferators, and no substantial change was brought about in the content of cholesterol by the treatments.

Effects on the Biosynthesis of PtdCho and PtdEtn in the Liver

The effects of tiadenol and DEHP on the activities of the enzymes that participate in the biosynthesis of PtdCho and PtdEtn were compared with the effect of clofibric acid (Table 3). Glycerol-3-phosphate acyltransferase activity in the rats was increased considerably by treatment with tiadenol (to the same extent as produced with clofibric acid), whereas DEHP induced this enzyme to a lesser extent compared with tiadenol and clofibric acid.

As regards the enzymes that participate in the synthesis of PtdCho, the three peroxisome proliferators increased slightly, but significantly, the activity of CTP:phosphocholine cytidylyltransferase when assayed in the presence of lipid vesicles. The activity of choline kinase was not affected by the three peroxisome proliferators. DEHP decreased the activity of CDP-choline:diacylglycerol cholinephosphotransferase in the presence of an exogenous substrate, although neither tiadenol nor clofibric acid altered this activity. The activity of PtdEtn *N*-methyltransferase was suppressed by all three peroxisome proliferators in the presence of an exogenous substrate.

The activity of CTP:phosphoethanolamine cytidylyltransferase was reduced markedly in common by the three peroxisome proliferators. In contrast, treatment of rats with tiadenol or DEHP significantly elevated the activity of PtdSer decarboxylase, as was also observed with clofibric acid treatment.

Effects on Molecular Species of PtdCho and PtdEtn in the Liver

Since the rat liver contains a diacyl form of PtdCho and PtdEtn, but neither alkylacyl nor alkenylacyl forms [24], the effects of the peroxisome proliferators on the composition of the molecular species of the diacyl form of PtdCho and PtdEtn were examined in the present study. Upon treatment of rats with tiadenol, DEHP, and clofibric acid, the composition of the molecular species of PtdCho in the liver was changed markedly (Fig. 2). Namely, the peroxisome proliferators strikingly increased the content of 16:0-18:1* PtdCho and conversely decreased the content of 18:0-18:2. Tiadenol and DEHP, but not clofibric acid, reduced the content of 16:0-18:2 PtdCho as well. Nevertheless, the proportion of the 16:0-18:2 species in PtdCho of clofibric acid-treated rats decreased to 71% of the control (11.89 ± 2.19 vs 16.80 ± 1.29 by mol%). The administration of tiadenol and DEHP considerably increased the content of 16:0-20:4 PtdCho but did not change the content of 18:0-20:4 PtdCho, whereas clofibric acid markedly decreased the content of 18:0-20:4 but not that of 16:0-20:4 PtdCho. The three peroxisome proliferators significantly affected the composition of the molecular species of diacyl-PtdEtn (Fig. 3). The marked increase in the contents of molecular species of 16:0-20:4 and 18:0-20:4 PtdEtn was common to the three peroxisome proliferators. In contrast to PtdCho, PtdEtn contains a 16:0-18:1 species at a lower proportion, and the increases induced by the three peroxisome proliferators in the content of 16:0-18:1 PtdEtn were not as great as those in PtdCho.

* The numerical designation of fatty acids indicates their chain lengths and numbers of double bonds: 16:0, palmitic acid; 18:0, stearic acid; 18:1, oleic acid; 18:2, linoleic acid; 20:4, arachidonic acid; and 22:6, docosahexaenoic acid. The use of a hyphen between two numerical designations indicates that a particular phosphoglyceride contains both fatty acids.

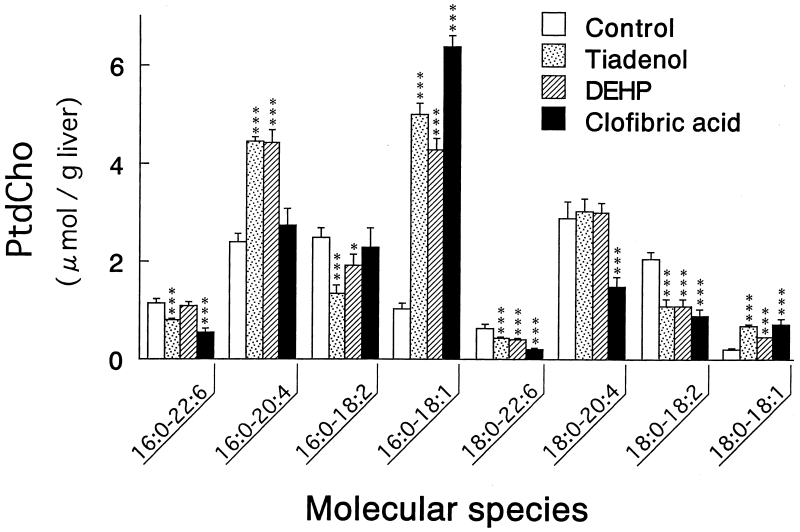

FIG. 2. Changes in the molecular species of hepatic diacyl-PtdCho by tiadenol, DEHP, and clofibric acid. Rats were fed a control diet or a diet containing 0.15% (w/w) tiadenol, 2% (w/w) DEHP, or 0.5% (w/w) clofibric acid for 7 days. Values are the means ± SD for four or five rats. Significantly different from control: (*) $P < 0.05$; and (***) $P < 0.001$.

Effects on Serum Lipid

Table 4 shows the effects of the three peroxisome proliferators on serum lipids. Serum concentrations of triacylglycerol and cholesterol were decreased markedly by tiadenol and DEHP, as well as by clofibric acid. The serum level of phospholipids was lowered considerably by the administration of the three peroxisome proliferators; the concentration of PtdCho, a major serum phospholipid, was reduced to approximately half that of the control. The concentrations of the molecular species of diacyl-PtdCho were affected individually by tiadenol, DEHP, or clofibric acid (Fig. 4). Serum concentrations of 16:0-18:2 and 18:0-18:2 PtdCho, which are originally major molecular species of PtdCho in serum, were decreased strikingly by the three peroxisome proliferators.

DISCUSSION

The present study revealed that tiadenol, DEHP, and clofibric acid in common increased considerably the contents of PtdCho and PtdEtn in rat liver regardless of their chemical structures. The underlying mechanism by which peroxisome proliferators induced metabolic alterations in the biosynthesis of PtdCho and PtdEtn, resulting in the elevation of hepatic levels of these phospholipids, is shown in Fig. 5. Glycerol-3-phosphate acyltransferase, the first key enzyme in the biosynthesis of glycerolipids, was induced by the three peroxisome proliferators in accordance with a previous study [25], suggesting an increased formation of all glycerolipids in the liver.

The three peroxisome proliferators increased the activity of CTP:phosphocholine cytidylyltransferase, the rate-limiting enzyme of PtdCho synthesis *de novo*, and suppressed PtdEtn *N*-methylation. In agreement with the generally accepted understanding of coordinate regulation of PtdCho synthesis *via* the CDP-choline pathway and PtdEtn *N*-methylation [26, 27], peroxisome proliferators, regardless of their structures, seem to increase the hepatic content of PtdCho by enhancing the synthesis *de novo*. These results are consistent with the findings of Mandla *et al.* [28], who

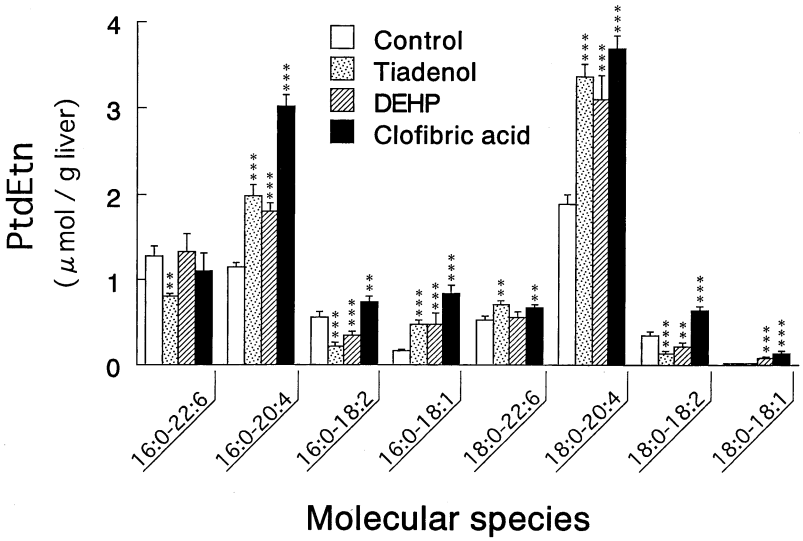

FIG. 3. Changes in the molecular species of hepatic diacyl-PtdEtn by tiadenol, DEHP, and clofibric acid. Rats were fed a control diet or a diet containing 0.15% (w/w) tiadenol, 2% (w/w) DEHP, or 0.5% (w/w) clofibric acid for 7 days. Values are the means ± SD for four or five rats. Significantly different from control: (**) $P < 0.01$; and (***) $P < 0.001$.

TABLE 4. Effects of tiadenol, DEHP, and clofibric acid on serum lipids

Treatment	Lipids (μmol/mL)			
	Phospholipids	PtdCho	Triacylglycerol	Cholesterol
Control	2.08 ± 0.15	1.58 ± 0.27	1.74 ± 0.14	1.93 ± 0.22
Tiadenol	1.47 ± 0.15*	0.78 ± 0.03*	0.41 ± 0.06*	1.19 ± 0.15*
DEHP	1.22 ± 0.07*	0.60 ± 0.06*	0.18 ± 0.03*	1.05 ± 0.11*
Clofibric acid	1.37 ± 0.07*	0.72 ± 0.09*	0.65 ± 0.20*	1.21 ± 0.10*

Rats were fed a control diet or a diet containing 0.15% (w/w) tiadenol, 2% (w/w) DEHP, or 0.5% (w/w) clofibric acid for 7 days. Values are the means ± SD for four or six rats.
*Significantly different from control: $P < 0.001$.

showed that clofibrate stimulates the incorporation of choline into PtdCho in cultured human fibroblasts. The present study revealed that the three peroxisome proliferators in common increased the hepatic content of the molecular species of PtdCho containing 18:1, especially 16:0-18:1 PtdCho. This marked increase seems to be due to the increased activity of *de novo* synthesis of PtdCho and to the enhanced supply of 18:1 produced by inducing stearoyl-CoA desaturase [29]. On the other hand, the three peroxisome proliferators lowered the hepatic proportion of 16:0-18:2 and 18:0-18:2 PtdCho, which are preferred sources of PtdCho for secretion into blood circulation [20, 30], leading to a decrease in the secretion of PtdCho from the liver. Thus, the peroxisome proliferators, regardless of their structures, appear to channel specific molecular species of PtdCho produced toward the biogenesis of organelles.

More than 40% of the phospholipid in peroxisomes has been shown to be PtdEtn [31]. This proportion is much higher compared with those in microsomes and mitochondria. Under the influence of peroxisome proliferators, therefore, hepatocytes must stimulate the formation of PtdEtn to proliferate peroxisomes. PtdEtn can be synthesized by three pathways, namely, the CDP-ethanolamine pathway, PtdSer decarboxylation, and the calcium-stimulated exchange of base [26, 27]; CTP:phosphoethanolamine cytidylyltransferase is regarded as the rate-limiting step of the CDP-ethanolamine pathway. The administration of clofibric acid to rats increased the hepatic concentration of PtdEtn; nevertheless, clofibric acid markedly inhibited the incorporation of ethanolamine into PtdEtn *in vivo* in rat liver [8] and *in vitro* in cultured human fibroblasts [28]. Moreover, the present study showed that the administration of tiadenol or DEHP strikingly suppressed the activity of CTP:phosphoethanolamine cytidylyltransferase, as well. Taken together, these results strongly suggest that the CDP-ethanolamine pathway is not responsible for the peroxisome proliferator-caused proliferation of organelles and hepatomegaly. In contrast, the three peroxisome proliferators increased the activity of PtdSer decarboxylase and decreased the activity of PtdEtn *N*-methyltransferase, suggesting that hepatocytes, in response to peroxisome proliferators, facilitate the formation of PtdEtn through the pathway of glycerol-3-phosphate → PtdCho → PtdSer → PtdEtn to increase the supply of PtdEtn for the proliferation of organelles. Analyses of molecular species of PtdEtn revealed that the three peroxisome proliferators increased the hepatic contents of 16:0-20:4 and 18:0-20:4 PtdEtn. These results are consistent with the findings of Kyrklund and Meijer [31], who demonstrated that treatment of rats with clofibrate increases the proportion of 20:4 in peroxisomal PtdEtn.

Clofibric acid is considered to be an active principle itself, and tiadenol and DEHP have been shown to be metabolized to the corresponding proximate forms that induce peroxisomal β-oxidation [32, 33]. The chemical structures of these active principles of the three peroxisome

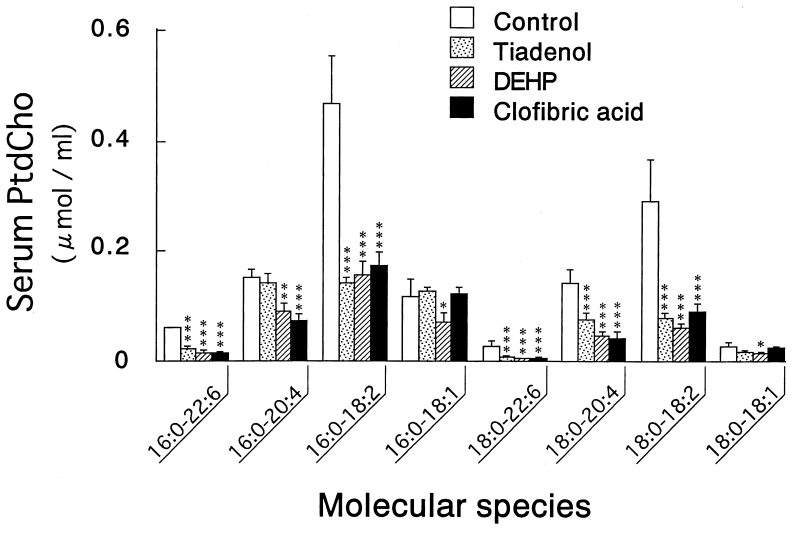

FIG. 4. Changes in the molecular species of serum diacyl-PtdCho by tiadenol, DEHP, and clofibric acid. Rats were fed a control diet or a diet containing 0.15% (w/w) tiadenol, 2% (w/w) DEHP, or 0.5% (w/w) clofibric acid for 7 days. Values are the means ± SD for four rats. Significantly different from control: (*) $P < 0.05$; (**) $P < 0.01$; and (***) $P < 0.001$.

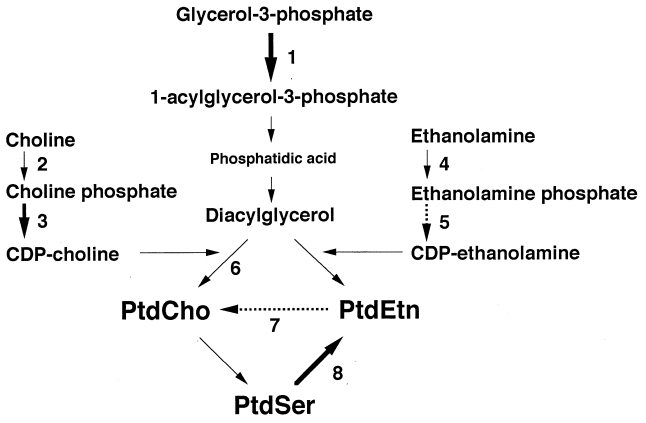

FIG. 5. Metabolic alterations by peroxisome proliferators of the biosynthesis of PtdCho and PtdEtn in the liver. The broad arrows indicate that the activities of the enzymes have been confirmed to be increased in common by the administration of tiadenol, DEHP, and clofibric acid to rats. The dotted arrows indicate that the activities of the enzymes have been confirmed to be suppressed in common by treatment of rats with the three peroxisome proliferators. Key: (1) glycerol-3-phosphate acyltransferase; (2) choline kinase; (3) CTP:phosphocholine cytidylyltransferase; (4) ethanolamine kinase; (5) CTP:phosphoethanolamine cytidylyltransferase; (6) CDP-choline:diacylglycerol cholinephosphotransferase; (7) PtdEtn N-methyltransferase; and (8) PtdSer decarboxylase.

proliferators, however, are very different from each other. Accordingly, the present results may support the conclusion that peroxisome proliferators in common, irrespective of their structures, increase the hepatic contents of PtdCho and PtdEtn by the same mechanism and that the increased formation of specific molecular species of PtdCho and PtdEtn is required to achieve the proliferation of organelles and hepatomegaly. However, it still remains to be investigated whether the expression of the genes of the enzymes that were examined in the present study is under the control of a peroxisome proliferator-activated receptor.

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