J Pharmacol Sci **111**, 201 – 210 (2009)

Journal of Pharmacological Sciences
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Full Paper

Peroxisome Proliferators Attenuate Free Arachidonic Acid Pool in the Kidney Through Inducing Lysophospholipid Acyltransferases

Tohru Yamazaki[1], Akihiko Hirose[2], Takeshi Sakamoto[1], Mari Okazaki[1], Atsushi Mitsumoto[3], Naomi Kudo[1], and Yoichi Kawashima[1,*]

[1]*Faculty of Pharmaceutical Sciences, Josai University, 1-1 Keyakidai, Sakado, Saitama 350-0295, Japan*
[2]*Division of Risk Assessment, National Institute of Health Sciences, 1-18-1 Kamiyoga, Setagaya-ku, Tokyo 158-8501, Japan*
[3]*Faculty of Pharmaceutical Sciences, Josai International University, 1 Gumyo, Togane, Chiba 283-8555, Japan*

Received May 30, 2009; Accepted August 25, 2009

Abstract. Attenuating effects of peroxisome proliferators on the concentration of free arachidonic acid by inducing 1-acyl-2-lysophospholipid acyltransferases in the kidney were studied. The administration of the three structurally dissimilar peroxisome proliferators, 2-(4-chlorophenoxy)-2-methylpropionic acid (clofibric acid), di(2-ethylhexyl)phthalate, and 2,2'-(decamethylenedithio)diethanol, to rats or mice considerably increased the activities of microsomal 1-acylglycerophosphoethanolamine acyltransferase (LPEAT), 1-acylglycerophosphoinositol acyltransferase (LPIAT), 1-acylglycerophosphoserine acyltransferase (LPSAT), and 1-acylglycerophosphocholine acyltransferase (LPCAT), and the mRNA level of LPCAT3, but not the mRNA level of LPCAT1, LPCAT4, or LPEAT1, in the kidney and the liver. The proportions of arachidonic acid in phospholipids in renal microsomes are rather high for the low proportion of arachidonic acid in free fatty acids in renal microsomes of control rats. The treatment of rats with clofibric acid attenuated the concentration and the proportion of free arachidonic acid to about a half; nevertheless the treatment lowered slightly the proportions of arachidonic acid in phospholipids other than phosphatidylcholine. These results indicate that peroxisome proliferators up-regulate the four 1-acyl-2-lysophospholipid acyltransferases of the kidney and, and the induced 1-acyl-2-lysophospholipid acyltransferases seem to play a physiologically crucial contribution in attenuating the pool of free arachidonic acid in the kidney.

Keywords: peroxisome proliferator, lysophospholipid acyltransferase, free arachidonic acid, kidney

Introduction

Since ethyl 2-(4-chlorophenoxy)-2-methylpropionic acid (clofibrate) was found to be a potent peroxisome proliferator (1), many attempts have been made to confirm the relationship between peroxisome proliferation and hypolipidemic action of the drug. Lazarow and de Duve showed that peroxisomes of rat liver contain fatty acid β-oxidation activity distinct from the mitochondrial one and that the activity of peroxisomal β-oxidation can be induced by the administration of clofibrate to rats (2). These findings stimulated the studies on peroxisome proliferators focusing upon the mechanism responsible for the inductions of the enzymes that compose the peroxisomal β-oxidation system. On the other hand, the administration of clofibrate to mice was shown to cause an increase in activity of glycerophosphate acyltransferase in hepatic microsomes (3). Differing from the peroxisomal enzymes, this enzyme is considered to participate in glycerolipid biosynthesis, which suggests that clofibrate affects lipid metabolism of animals through inducing not only the enzymes related to lipid degradation, but also the enzymes involved in lipid biosynthesis. In fact, peroxisome proliferators were demonstrated to induce many other enzymes such as stearoyl-CoA desaturase (4, 5), palmitoyl-CoA elongase (6 – 8), acyl-CoA synthetase (7, 8), 1-acylglycerophosphate acyltransferase

*Corresponding author. ykawash@josai.ac.jp
Published online in J-STAGE on October 6, 2009 (in advance)
doi: 10.1254/jphs.09162FP

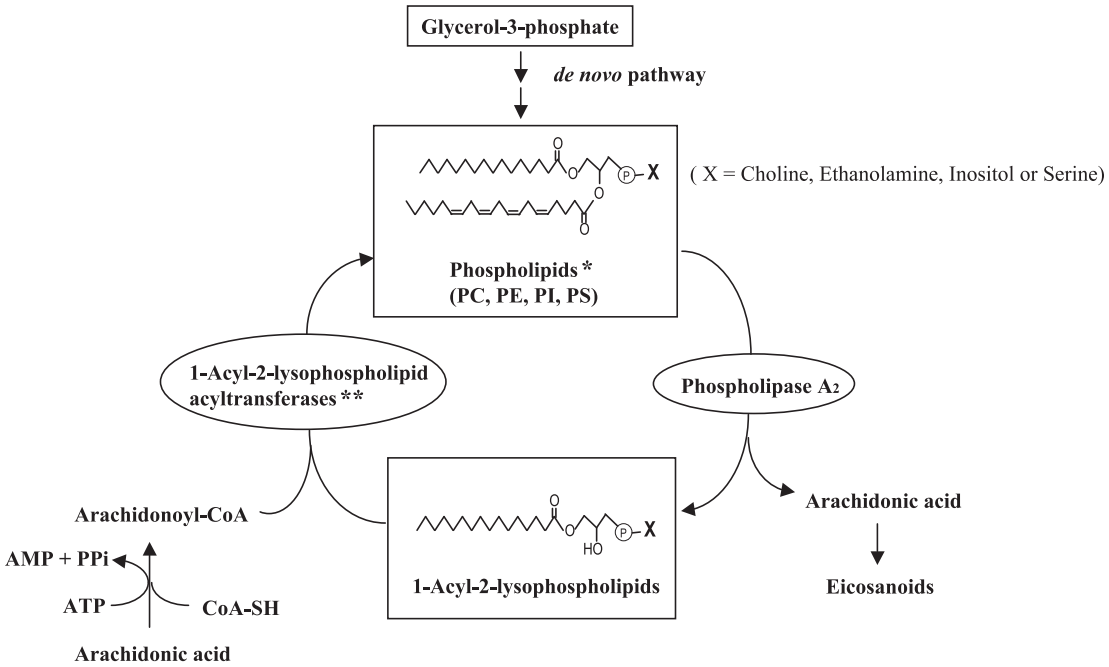


Fig. 1. The remodeling pathway of phospholipid synthesis. *PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine. **LPCAT, 1-acyl-2-lysophosphatidylcholine acyltransferase; LPEAT, 1acyl-2-lysophosphatidyl-ethanolamine acyltransferase; LPIAT, 1-acyl-2-lysophosphatidylinositol acyltransferase; LPSAT, 1-acyl-2-lysophosphatidylserine acyltransferase. AMP, adenosine monophosphate; ATP, adenosine triphosphate; PPi, inorganic pyrophosphate.

(8), CoA-dependent transacylase (9), and 1-acylglycerophosphocholine acyltransferase (LPCAT) (10, 11). These enzymes are considered to participate in modification of the acyl moiety of glycerolipids. In contrast with LPCAT, 2-acylglycerophosphocholine acyltransferase was not induced by peroxisome proliferators (10). The primary physiological role of LPCAT is considered to generate phosphatidylcholine (PC) having an unsaturated fatty acid at the *sn*-2 position (12). These findings suggested that peroxisome proliferators cause alterations in acyl composition of PC. In fact, the previous study showed that the treatment of rats with 2-(4-chlorophenoxy)-2-methylpropionic acid (clofibric acid) caused a marked alteration in acyl composition of PC (8) and, consequently, in the composition of molecular species of PC in the liver (13). Moreover, the clofibric acid–caused changes in acyl composition of hepatic PC could be elucidated mainly as the result of the inductions of stearoyl-CoA desaturase, palmitoyl-CoA elongase, and LPCAT in the liver (8, 14, 15). Similar alterations in acyl composition were observed in other liver phospholipids such as phosphatidylethanolamine (PE), phosphatidylinositol (PI) and phosphatidylserine (PS) (16). When the response of organs of rats to peroxisome proliferators was estimated with the induction of peroxisomal β-oxidation, the kidney was the second most sensitive organ among the organs tested (liver, kidney, intestinal mucosa, brain, testis, lung, heart, spleen, and skeletal muscle) next to the liver (17). Moreover, the treatment of rats with peroxisome proliferators induced LPCAT in the kidney to an extent comparable to that in the liver (17). These findings suggest the possibility that peroxisome proliferators induce not only LPCAT, but also 1-acylglycerophosphoethanolamine acyltransferase (LPEAT), 1-acylglycerophosphoinositol acyltransferase (LPIAT), and 1-acylglycerophosphoserine acyltransferase (LPSAT) in the kidney and liver. In addition to the role in remodeling the acyl moiety of phospholipids, 1-acyl-2-lysophospholipid acyltransferases are thought to play an important role in regulating the cellular pool of free arachidonic acid (18, 19) (Fig. 1). Therefore, if peroxisome proliferators upregulate the activities of 1-acyl-2-lysophospholipid acyltransferases, peroxisome proliferators must affect the formation of eicosanoids through reducing cellular concentration of free arachidonic acid, leading to alteration in renal functions. Moreover, we noted the previous findings that renal phospholipids contain arachidonic acid as the acyl moiety in high proportions (16). Since little is known about the effects of peroxisome proliferators on 1-acyl-2-lysophospholipid acyltransferases other than LPCAT, it is deemed necessary to study whether peroxisome proliferators also induce the other lysophospholipid acyltransferases. The aim of the present study is to

answer the questions of whether peroxisome proliferators induce LPEAT, LPIAT, and LPSAT in the kidney and liver and whether peroxisome proliferators attenuate the concentration of free arachidonic acid in the kidney.

Materials and Methods

Materials

[1-^{14}C]Oleoyl-CoA (57.0 Ci/mol) was purchased from PerkinElmer Japan Co. (Tokyo). Oleoyl-CoA, palmitoyl-CoA, clofibric acid, and bovine serum albumin were purchased from Sigma (St. Louis, MO, USA); 2,2'-(decamethylenedithio)diethanol (tiadenol), from Aldrich Chemical Co. (Milwaukee, WI, USA); di(2-ethylhexyl) phthalate (DEHP), from Tokyo Chemical Industry Co. (Tokyo); 1-acylglycerophosphocholine from Avanti Polar Lipids, Inc. (Alabaster, AL, USA); 1-acylglycerophosphoethanolamine, 1-acylglycerophosphoinositol, and 1-acylglycerophosphoserine, from Serdary Research Laboratory (London, Ontario, Canada); CoA and NAD^+, from Oriental Yeast Co. (Tokyo). All other chemicals were of analytical grade.

Animals

All animal studies complied with the recommendations of the Institutional Board for Animal Studies, Josai University. Male Wistar rats aged 4 weeks and male ddY mice aged 6 weeks were obtained from SLC (Hamamatsu). Animals were fed on a standard diet (CE-2; Clea Japan, Tokyo) ad libitum and allowed free access to water. After acclimatization for 1 week, the rats were divided into four groups and were fed on the standard diet or a diet containing one of the following: 0.5% (w/w) clofibric acid, 0.5% (w/w) tiadenol, or 0.5% (w/w) DEHP for 7 days. Mice were divided into three groups and were fed on the standard diet or a diet containing 0.5% (w/w) clofibric acid or 0.5% (w/w) tiadenol for 7 days. After blood was withdrawn from the descending vena cava under diethyl ether anesthesia, kidneys and livers were excised. Microsomes from the kidney and the liver were prepared by differential centrifugations as described previously (13). The concentrations of protein were determined by the method of Lowry et al. (20) with bovine serum albumin as a standard.

Enzyme assays

The activities of 1-acyl-2-lysophospholipid acyltransferases were measured by the optical assays that are based on the reaction of liberated coenzyme A with 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) or by the method utilizing [1-^{14}C]oleoyl-CoA. The reaction mixture for the LPEAT assay contained 10 nmol (for the liver) or 2.5 – 5 nmol (for the kidney) oleoyl-CoA, 40 nmol 1-acylglycerophosphoethanolamine, 186 nmol sodium deoxycholate, 1 μmol DTNB, 100 μmol Tris-HCl buffer (pH 7.4), and 100 μg microsomal protein in a final volume of 1 ml. 1-Acylglycerophosphoethanolamine was dissolved in the buffer with sodium deoxycholate because of its low solubility. The reaction mixture for LPIAT assay contained 10 nmol (for the liver) or 5 nmol (for the kidney) oleoyl-CoA, 50 nmol 1-acylglycerophosphoinositol, 1 μmol DTNB, 100 μmol Tris-HCl buffer (pH 7.4), and 100 μg microsomal protein in a final volume of 1 ml. The reaction mixture for LPSAT assay contained 10 nmol (for the liver) or 2.5 nmol (for the kidney) oleoyl-CoA, 40 nmol 1-acylglycerophosphoserine, 1 μmol DTNB, 100 μmol Tris-HCl buffer (pH 7.4), and 100 μg microsomal protein in a final volume of 1 ml. The reaction mixture for LPCAT assay contained 20 – 30 nmol oleoyl-CoA, 150 nmol 1-acylglycerophosphocholine, 1 μmol DTNB, 100 μmol Tris-HCl buffer (pH 7.4), and 100 μg microsomal protein in a final volume of 1 ml. It should be noted that the optimal concentrations of oleoyl-CoA for LPEAT, LPIAT, and LPSAT in the kidney were much lower than those in the liver and that, in contrast to the case of oleoyl-CoA, no substantial difference was found in the optimal concentrations of 1-acyl-2-lysophospholipids between the kidney and the liver (not shown). After the preincubation for 2 min in the absence of oleoyl-CoA, the incubation was initiated by adding oleoyl-CoA, and the increase in absorbance at 412 nm was followed at 30°C. The control value without lysophospholipid was subtracted to obtain the net acyl transfer rate. When [1-^{14}C]oleoyl-CoA was used as a substrate, DTNB was omitted from the reaction mixture. The radioactive reaction products were extracted by the method of Bligh and Dyer (21) and separated by thin-layer chromatography on silica gel G plates (Merck, Darmstadt, Germany), which were developed with a solvent system comprised of chloroform / methanol / acetic acid / water (100:75:7:4, v/v) (22). The areas corresponding to individual phospholipids were scraped off the plate and transferred to tubes. The phospholipids were extracted from the silica gel with chloroform / methanol / 0.1 M HCl (4:4:1, v/v) (23). An aliquot of the extract was transferred to a counting vial and taken to dryness. The residue was dissolved in a toluene-Triton X-100 (2:1, v/v)–based scintillator, and the radioactivity was measured. Peroxisomal β-oxidation was measured by the method of Lazarow and de Duve (2) using palmitoyl-CoA as a substrate and homogenates of the kidney and the liver as enzyme sources, as described previously (11).

Table 1. Sequences of primers used for real-time PCR

	Primer sequence (5' – 3')	Product size (bp)	Accession number
AOX*			
mouse	F: TTCGTGCAGCCAGATTGGTAG (1807 – 1827) R: CGGCTTTGTCTTGAATCTTGG (1992 – 1972)	186	NM_015729
rat	(F: 1539 – 1560, R: 1745 – 1724)	186	NM_017340
LPCAT1			
mouse	F: CGTGAATATGTGGTCGCCTTG (1204 – 1224) R: ATGCTGCCATCCTCAGGAGAT (1308 – 1287)	106	NM_145376
rat	F: CTCCTGAGGATGGCAGCATAGA (1426 – 1447) R: TCAATAGCCTGGAACAAGTCGG (1528 – 1507)	105	NM_001100735
LPCAT3*			
mouse	F: TTTCTGGTTCCGCTGCATGT (796 – 815) R: CCGACAGAATGCACACTCCTTC (896 – 875)	103	AB294194
rat	(F: 845 – 864, R: 945 – 924)	103	BC089869
LPCAT4*			
mouse	F: TTCGGTTTCAGAGGATACGACAA (907 – 929) R: AATGTCTGGATTGTCGGACTGAA (1094 – 1072)	190	NM_001083341
rat	(F: 911 – 933, R: 1098 – 1076)	190	NM_001108016
LPEAT1*			
mouse	F: CTGAAATGTGTGTGCTATGAGCG (1066 – 1088) R: TGGAAGAGAGGAAGTGGTGTCTG (1243 – 1221)	178	AB297382
rat	(F: 1251 – 1273, R: 1428 – 1406)	180	NM_001109120
β-Actin			
mouse	F: CGTGCGTGACATCAAAGAGAA (704–725) R: AGGAAGAGGATGCGGCAGT (802–783)	80	NM_007393
rat	F: TGCAGAAGGAGATTACTGCC (2820–2840) R: CGCAGCTCAGTAACAGTCC (3174–3155)	220	V01217

*The primers can amplify both mouse and rat mRNAs.

RNA extraction and real-time quantitative polymerase chain reaction (PCR)

Total RNA was extracted from the kidney and the liver using QIAzol reagent and an RNeasy kit (QIAGEN, Hilden, Germany). Complementary DNA (cDNA) was synthesized from 500 ng of total RNA with avian myeloblastosis virus reverse transcriptase (Takara, Tokyo). PCR amplification was carried out by using QuantiTect SYBR green PCR master mix (QIAGEN, Hilden, Germany). The amplification and detection were performed with an iCycler IQ real-time detection system (Bio-Rad, Hercules, CA, USA). Primer express software ver. 2.0 (Applied Biosystems, Foster City, CA, USA) was utilized for the design of the primers. The sequences of primers used in this study are listed in Table 1. To demonstrate the specificity of the PCR product, melting curve analysis was performed after PCR, and each curve was confirmed to be as a single peak (data not shown). The thermal cycling program was as follows: 15 min denaturation step at 95°C followed by 40 cycles of 15 s denaturation at 94°C, 30 s annealing at 57°C, and 30 s extension at 72°C. Changes in gene expression were calculated by using the comparative threshold cycle (Ct) method. Ct values were first normalized by subtracting the Ct value obtained from *β*-actin.

Lipid analyses

After the addition of heptadecanoic acid as an internal standard for measuring free fatty acids, total lipid was extracted from a slightly acidified renal microsomal suspension by the method of Bligh and Dyer (21). Free fatty acid was separated by thin-layer chromatography on silica gel G plates, and the plates were developed by *n*-hexane / diethyl ether / acetic acid (80:30:1,v/v). Phospholipids were separated by thin-layer chromatography on silica gel G plates, which were developed with a solvent system comprised of chloroform / methanol / acetic acid / water (100:75:7:4, v/v) (22). After visualizing by spraying 0.001% (w/v) primuline

Table 2. Effects of peroxisome proliferators on the activities of 1-acyl-2-lysophospholipid acyltransferases in microsomes in the kidney of rats

Animal groups	LPEAT	LPIAT	LPSAT	LPCAT	Peroxisomal β-oxidation
			(nmol/min per mg protein)		
Control	2.2 ± 0.1^{a}	11.3 ± 1.5^{a}	6.3 ± 0.9^{a}	32.6 ± 1.8^{a}	2.7 ± 0.4^{a}
Clofibric acid	15.0 ± 1.2^{b}	33.4 ± 2.0^{b}	26.0 ± 3.9^{b}	213.5 ± 9.6^{b}	6.1 ± 0.6^{b}
Tiadenol	10.3 ± 0.6^{c}	24.3 ± 2.0^{c}	26.5 ± 3.1^{b}	165.8 ± 8.5^{c}	12.2 ± 1.1^{c}
DEHP	9.0 ± 0.7^{c}	18.1 ± 0.8^{d}	22.4 ± 3.2^{b}	138.6 ± 6.6^{d}	6.3 ± 0.5^{b}

Rats were fed on a control diet or a diet containing 0.5% (w/w) clofibric acid, 0.5% (w/w) tiadenol, or 0.5% (w/w) DEHP for 7 days. Values each represent the mean ± S.D. for three or four rats. ^{a,b,c,d}Differences in vertical means without a common superscript are statistically significant ($P<0.05$). If there is no superscript, the differences in the means are not significant ($P>0.05$).

Table 3. Effects of peroxisome proliferators on the activities of 1-acyl-2-lysophospholipid acyltransferases in microsomes in the liver of rats

Animal groups	LPEAT	LPIAT	LPSAT	LPCAT	Peroxisomal β-oxidation
			(nmol/min per mg protein)		
Control	7.8 ± 0.6^{a}	29.8 ± 3.5^{a}	19.0 ± 0.5^{a}	64.9 ± 5.8^{a}	3.1 ± 0.2^{a}
Clofibric acid	22.8 ± 1.5^{b}	72.6 ± 13.1^{b}	66.4 ± 3.3^{b}	281.4 ± 26.3^{b}	42.9 ± 3.3^{b}
Tiadenol	30.6 ± 1.3^{c}	54.6 ± 7.5^{bc}	73.7 ± 19.3^{b}	283.8 ± 7.9^{b}	60.1 ± 1.6^{c}
DEHP	17.1 ± 1.5^{d}	40.8 ± 5.5^{ac}	78.6 ± 9.9^{b}	206.6 ± 13.1^{c}	51.7 ± 4.9^{d}

Rats were fed on a control diet or a diet containing 0.5% (w/w) clofibric acid, 0.5% (w/w) tiadenol, or 0.5% (w/w) DEHP for 7 days. Values each represent the mean ± S.D. for four rats. ^{a,b,c,d}Differences in vertical means without a common superscript are statistically significant ($P<0.05$). If there is no superscript, the differences in the means are not significant ($P>0.05$).

in acetone, the regions on the plates that corresponded to individual lipids were scraped off and transferred to tubes. The lipids were extracted from silica gel with chloroform / methanol / 0.1 M HCl (4:4:1, v/v) (23). Methyl esters of fatty acids were prepared from each extract using boron trifluoride in methanol for free fatty acids and sodium methoxide in methanol for phospholipids. All solvents employed for lipid analyses contained 0.005% (w/v) butylated hydroxytoluene. The amounts and composition of the fatty acid methyl esters were determined by gas-liquid chromatography as described previously (23).

Statistical analyses

Analysis of variance was used to test significant differences between groups. Where differences were significant, the statistical significance ($P<0.05$) between any two means was determined using Scheffé's multiple range test as a *post-hoc* analysis (Stat-View; SAS Institute, Cary, NC, USA). The statistical significance between clofibric acid–treated and untreated rats was analyzed by Student's *t*-test after the *F*-test for two means.

Results

Upregulation by peroxisome proliferators of 1-acyl-2-lysophospholipid acyltransferases in the kidney and the liver of rats and mice

The treatment of rats with clofibric acid markedly induced the activities of LPEAT, LPIAT, LPSAT, and LPCAT in the kidney (Table 2). The extents of the increases in the activities of LPEAT, LPIAT, LPSAT, and LPCAT were 6.8, 3.0, 4.1, and 6.5 times, respectively, over the controls. To examine whether the increases in the activities of the four lysophospholipid acyltransferases are related to the ability of clofibric acid to proliferate peroxisomes, two other peroxisome proliferators structurally dissimilar to clofibric acid were administered to rats, and the responses of these acyltransferases to the peroxisome proliferators were examined. For this purpose, tiadenol and DEHP were chosen. The treatment of rats with DEHP or tiadenol considerably increased the activities of the four 1-acyl-2-lysophospholipid acyltransferases in the kidney, as was observed with clofibric acid (Table 2). Similarly, the effects of the three structurally dissimilar peroxisome proliferators on microsomal LPEAT, LPIAT, LPSAT, and LPCAT in the liver of rats were examined (Table 3), and the results showed that the increases in the

Table 4. Effects of peroxisome proliferators on the activities of 1-acyl-2-lysophospholipid acyltransferases in microsomes of the kidney and the liver of mice

Animal groups	LPEAT	LPIAT	LPSAT	LPCAT	Peroxisomal β-oxidation
			(nmol/min per mg protein)		
Kidney					
Control	3.9 ± 1.0[a]	19.6 ± 2.2[a]	13.4 ± 1.4[a]	57.6 ± 6.8[a]	5.2 ± 0.9[a]
Clofibric acid	8.7 ± 1.7[b]	26.2 ± 2.2[b]	32.7 ± 3.2[b]	131.2 ± 7.2[b]	11.6 ± 1.4[b]
Tiadenol	13.4 ± 1.9[c]	27.1 ± 0.7[b]	27.1 ± 0.5[b]	131.5 ± 17.2[b]	12.2 ± 1.1[b]
Liver					
Control	9.1 ± 0.6[a]	30.3 ± 6.1[a]	21.0 ± 2.3[a]	64.8 ± 5.5[a]	4.1 ± 0.5[a]
Clofibric acid	21.5 ± 4.2[b]	57.8 ± 3.5[b]	50.4 ± 10.0[b]	185.4 ± 17.0[b]	53.6 ± 8.2[b]
Tiadenol	25.1 ± 3.5[b]	79.6 ± 11.9[c]	60.0 ± 6.8[b]	314.4 ± 34.9[c]	79.6 ± 4.8[c]

Mice were fed on a control diet or a diet containing 0.5% (w/w) clofibric acid or 0.5% (w/w) tiadenol for 7 days. Values each represent the mean ± S.D. for four or six mice. [a,b,c]Differences in vertical means without a common superscript are statistically significant ($P<0.05$). If there is no superscript, the differences in the means are not significant ($P>0.05$).

activities of these four lysophospholipid acyltransferases in the liver are comparable to those in the kidney. To examine the inducing effects of peroxisome proliferators on 1-acyl-2-lysophospholipid acyltransferases in the kidney and the liver of mice, clofibric acid and tiadenol were administered to mice and the activities of LPEAT, LPIAT, LPSAT, and LPCAT were measured. In both the kidney and the liver, LPEAT, LPSAT, and LPCAT were considerably induced by either clofibric acid or tiadenol (Table 4). These inductions of 1-acyl-2-lysophospholipid acyltransferases were accompanied by an increase in the activity of peroxisomal β-oxidation in the kidney and the liver of both rats and mice (Tables 2 – 4). The induced activities of peroxisomal β-oxidation in the kidney of rats and mice were far less than those in the liver of rats and mice (Tables 2 – 4).

Effects of clofibric acid on the levels of mRNA encoding 1-acyl-2-lysophospholipid acyltransferases in the kidney and the liver

The effect of clofibric acid on levels of mRNAs encoding the 1-acyl-2-lysophospholipid acyltransferases and peroxisomal acyl-CoA oxidase (AOX) that were constitutively expressed in the kidney was evaluated with quantitative reverse transcription PCR (RT-PCR). mRNA levels of LPCAT3 and AOX were significantly increased in the kidney by the treatment of rats with clofibric acid, whereas those of LPEAT1, LPCAT1, and LPCAT4 were not affected (Fig. 2A). The mRNA levels of AOX and LPCAT3 were significantly increased in the liver of rats treated with clofibric acid, whereas those of LPEAT1, LPCAT1, and LPCAT4 were not affected (Fig. 2B). Quantitative RT-PCR analysis showed that mRNA levels of LPCAT3 and AOX were significantly

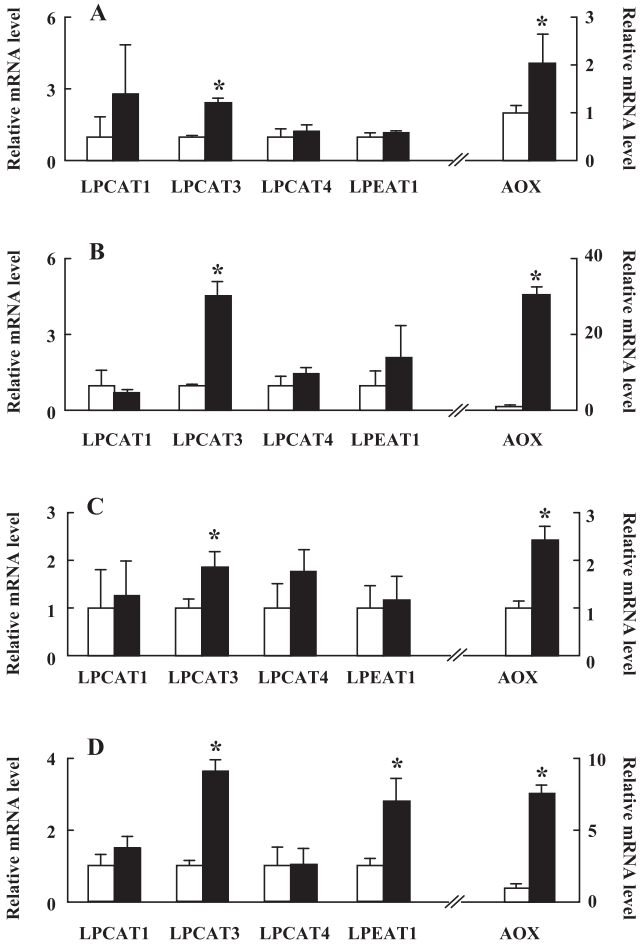


Fig. 2. Effects of clofibric acid on mRNA levels of LPCAT1, LPCAT3, LPCAT4, and LPEAT1. A, rat kidney; B, rat liver: C, mouse kidney; D, mouse liver. Open column, control; closed column, clofibric acid. Rats and mice were fed on a control diet or a diet containing 0.5% (w/w) clofibric acid for 7 days. Values are each the mean ± S.D. for four rats. *Significantly different from the control at $P<0.05$.

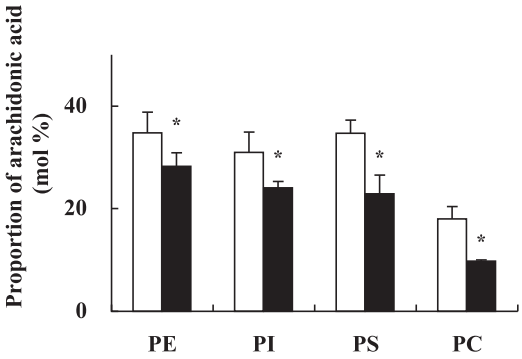


Fig. 3. Effects of clofibric acid on the proportions of arachidonic acid in microsomal phospholipids of the kidney. Open column, control; closed column, clofibric acid. Rats were fed on a control diet or a diet containing 0.5% (w/w) clofibric acid for 7 days. Values are each the mean ± S.D. for three rats. *Significantly different from control at $P<0.05$.

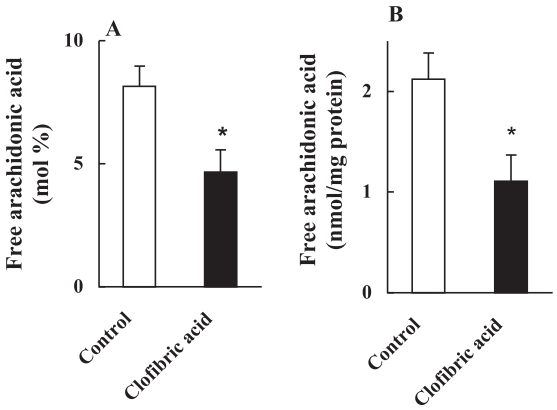


Fig. 4. Reducing effects of clofibric acid on the proportions and the concentrations of free arachidonic acid in microsomes of the kidney. A, proportion; B, concentration. Open column, control; closed column, clofibric acid. Rats were fed on a control diet or a diet containing 0.5% (w/w) clofibric acid for 7 days. Values are each the mean ± S.D. for three rats. *Significantly different from the control at $P<0.05$.

elevated by the treatment of mice with clofibric acid in the kidney and the liver (Fig. 2: C and D). mRNA expression of LPEAT1 was also increased in the liver, but not in the kidney of mice (Fig. 2: C and D).

Attenuation of concentration of free arachidonic acid in the kidney

The proportions of arachidonic acid in phospholipids in renal microsomes of control rats ranged from 18% to 35% (Fig. 3), despite the proportion of arachidonic acid in free fatty acids in renal microsomes being 8.1% (Fig. 4A). The administration of clofibric acid to rats decreased slightly the proportions of arachidonic acid in PE and PI, reduced moderately that in PS, and substantially lowered that in PC in microsomes of the kidney (Fig. 3). The treatment of rats with clofibric acid reduced the proportion and the concentration of free arachidonic acid in renal microsomes to 52% and 57%, respectively, of those in the controls (Fig. 4).

Discussion

The present study showed that the treatments of rats and mice with peroxisome proliferators, regardless of their structure, caused increases in the activities of LPEAT, LPIAT, and LPSAT in microsomes of both the kidney and the liver. Peroxisome proliferators have been demonstrated to induce many enzymes and proteins (24) that participate in degradation and biosynthesis of lipids, including AOX (25), acyl-CoA synthetase (7, 8), stearoyl-CoA desaturase (4, 5), lipoprotein lipase (26), fatty acid binding protein (27), and LPCAT (10, 11). Most of them have been proved to be induced by the action of peroxisome proliferators through peroxisome proliferator-activated receptor α (PPARα) (24, 28), which belongs to the steroid / thyroid / retinoic acid–receptor super family and acts as a ligand-dependent transcription factor. To our knowledge, however, neither LPCAT, LPEAT, LPIAT, nor LPSAT have been purified from either the kidney or the liver, and the detailed mechanisms responsible for the inductions of 1-acyl-2-lysophospholipid acyltransferases have not been clarified yet. Deka et al. observed the activities of LPEAT, LPIAT, and LPSAT disappearing during the course of the purification of LPCAT from bovine brain (29). Sanjanwala et al. showed that LPIAT was chromatographically separated from LPCAT, when purified from bovine heart microsomes (30). Yamashita et al. showed that the partial purified LPIAT from rat liver microsomes contained the activities of neither LPCAT nor LPEAT (31). These findings suggest that multiple forms of enzymes may exist in different tissues with different substrate specificities toward fatty acyl-CoAs and 1-acyl-2-lysophospholipids. However, attempts to purify the enzymes have not been successful largely due to the fact that the activities of 1-acyl-2-lysophospholipid acyltransferases were mostly lost during solubilization and further purification. Recently, through genomic efforts, the cloning of several distinctive 1-acyl-2-lysophospholipid acyltransferases was reported (32 – 35). LPCAT1 was demonstrated to have a preference for saturated fatty acyl-CoAs and was predominantly expressed in the lung (32, 33). The recent studies showed that LPCAT3 was primarily responsible for hepatic LPCAT with substrate specificities toward unsaturated fatty acyl-CoAs and 1-acylglycerophos-

phocholine (34, 35) and the possibility that the expression of LPCAT3 is regulated in a PPARα-dependent fashion (34). Our previous study showed that the administration of peroxisome proliferators to rats induces microsomal LPCAT in the liver, kidney, and the intestine, but not in the lung (17). Moreover, the present study showed that clofibric acid, a typical peroxisome proliferator, upregulated the expression of LPCAT3, but neither LPCAT1 nor LPCAT4, in the kidney of rats and mice. Therefore, the increase in the activities of LPCAT by clofibric acid in the kidney and the liver is possibly due to upregulation of LPCAT3. Very interestingly, LPCAT3 shows activities of LPEAT and LPSAT in addition to LPCAT activity (35). Therefore, the increase in the activities of LPEAT and LPSAT by clofibric acid is suggested to be at least partly due to upregulation of LPCAT3. At present, no genomic information is available about LPIAT. The mRNA expression of LPEAT1 was increased in the liver of mice, but not in either the kidney or the organs of rats, by the treatment with clofibric acid. It seems likely that there is a species difference in the induction of LPEAT1 in response to clofibric acid. However, further studies are required to elucidate the mechanism underlying the species and organ-specific induction.

The most important role of 1-acyl-2-lysophospholipid acyltransferases to be noted is that these enzymes participate in remodeling of pre-existing phospholipid molecules. Namely, phospholipids are produced *de novo* and then remodeled. The remodeling pathway involves deacylation of phospholipids followed by reacylation of lysophospholipids with acyl-CoAs to attain the proper fatty acids within phospholipids. Physiological significance of LPCAT and LPEAT for maintaining the asymmetry of the acyl group of PC and PE, respectively, has been studied well (12). Compared with LPCAT and LPEAT, less information is available about LPIAT and LPSAT. Hepatic microsomes of rats have been shown to contain the activities of LPIAT and LPSAT, and these enzymes are considered to participate in maintaining specific molecular species of PI and PS (36, 37). The previous studies showed that hepatic LPCAT, which is induced by clofibric acid, plays a crucial role in remodeling the acyl composition of the *sn*-2 position of PC (8) and that the induction of LPCAT consequently leads to a striking alteration in the composition of molecular species of PC (13). It seems possible, therefore, that the inductions of LPEAT, LPIAT, and LPSAT by peroxisome proliferators cause the changes in acyl composition of PE, PI, and PS, respectively, in the kidney and the liver. The changes were observed in rats being treated with clofibric acid (16).

The previous studies proposed the possibility that the inhibition of LPCAT enlarges the cellular pool of free arachidonic acid available for the formation of eicosanoids in macrophages and platelets (18, 19). Conversely, the present study showed that the proportions of arachidonic acid in PE, PI, PS, and PC were rather high for the low proportion of arachidonic acid in free fatty acids in renal microsomes of control rats and that, moreover, the treatment of rats with clofibric acid considerably reduced the proportion and the concentration of free arachidonic acid in renal microsomes by inducing the four 1-acyl-2-lysophospholipid acyltransferases; nevertheless, the proportions of arachidonic acid in PE, PI, and PS were kept substantially high. Our present results support the previous idea that 1-acyl-2-lysophospholipid acyltransferases are crucial factors regulating the cellular concentration of free arachidonic acid (18, 19).

Our previous study showed that the treatment of rats with clofibric acid strikingly reduced prostaglandin E_2 formation in kidney slices (38). Consequently, the present results suggest that peroxisome proliferators make an important contribution in attenuating the pool of free arachidonic acid by inducing 1-acyl-2-lysophospholipid acyltransferases, resulting in the decrease in eicosanoid productions in the kidney. It is unlikely that peroxisomal β-oxidation regulates the concentration of free arachidonic acid in the kidney because the induced activities of peroxisomal β-oxidation by peroxisome proliferators in the kidney were substantially lower than those in the liver. It is considered that eicosanoids, which are formed from arachidonic acid, regulate renal blood flow and glomerular filtration (39). It seems likely, therefore, that 1-acyl-2-lysophospholipid acyltransferases modulate renal hemodynamics through regulating the pool of free arachidonic acid in the kidney.

In summary, the present study showed that peroxisome proliferators increase the activities of LPEAT, LPSAT, LPIAT, and LPCAT in the kidney and the liver and that the induction of these 1-acyl-2-lyophospholipid acyltransferases reduces the pool of free arachidonic acid in the kidney. These results suggest that 1-acyl-2-lyophospholipid acyltransferases modulate renal hemodynamics through regulating the formation of eicosanoids from arachidonic acid.

Acknowledgment

This research was supported by a Grant-in-Aid for Scientific Research (C) from the Ministry of Education, Culture, Sports, Science, and Technoloogy, Japan.

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