Sex-related differences in the enhancing effects of perfluoro-octanoic acid on stearoyl-CoA desaturase and its influence on the acyl composition of phospholipid in rat liver

Comparison with clofibric acid and tiadenol

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The effects of the peroxisome proliferators clofibric acid (*p*-chlorophenoxyisobutyric acid), tiadenol [2,2'-(decamethylenedithio)diethanol] and perfluoro-octanoic acid (PFOA) on hepatic stearoyl-CoA desaturation in male and female rats were compared. Treatment of male rats with the three peroxisome proliferators increased markedly the activity of stearoyl-CoA desaturase. Administration of clofibric acid or tiadenol to female rats increased greatly the hepatic activity of stearoyl-CoA desaturase, the extent of the increases being slightly less pronounced than those of male rats. In contrast with the other two peroxisome proliferators, however, PFOA did not change the activity of stearoyl-CoA desaturase in female rats. Hormonal manipulations revealed that this sex-related difference in the effect of PFOA on stearoyl-CoA desaturase activity is strongly dependent on testosterone. The increase in stearoyl-CoA desaturase activity by peroxisome proliferators was not accompanied by any notable increases in the microsomal content of cytochrome *b*₅ or the activity of NADH: cytochrome *b*₅ reductase. The administration of the peroxisome proliferators greatly altered the acyl composition of hepatic phosphatidylcholine and phosphatidylethanolamine (namely the proportions of C_{18:1} and C_{20:3, n-9} fatty acids increased in both phospholipids), and the alterations were partially associated with the increase in stearoyl-CoA desaturase activity.

INTRODUCTION

Stearoyl-CoA desaturase is an enzyme introducing a double bond at the C-9 position of stearoyl-CoA to form oleoyl-CoA [1]. Extensive studies revealed that the activity of stearoyl-CoA desaturase is regulated by many physiological factors, especially the hormonal and nutritional state of the animal [2–7]. Stearoyl-CoA desaturase is one of the predominant factors controlling the acyl composition of hepatic lipid [7]. In comparison with numerous studies on the hormonal and nutritional regulation of stearoyl-CoA desaturase, less is known about effects of foreign compounds on this enzyme. Most chemicals tested were shown to be inhibitory to stearoyl-CoA desaturase [8–10]. Our previous studies, however, first showed that clofibric acid (*p*-chlorophenoxyisobutyric acid), a hypolipidaemic drug, increased markedly the activity of this enzyme in rat liver [11] and that the increase in stearoyl-CoA desaturase activity by clofibric acid caused great alterations of acyl composition of hepatic lipid [12]. Subsequent studies [13,14] showed that acetylsalicylic acid, di-(2-ethylhexyl) phthalate, dibutyl phthalate, di-(2-ethylhexyl) adipate and tiadenol [2,2'-(decamethylenedithio)diethanol] also increased the activity of stearoyl-CoA desaturase. A common property among these chemicals is to be a peroxisome proliferator [15].

A number of compounds have been confirmed to be peroxisome proliferators [15]. Recently perfluorinated

carboxylic acids, such as PFOA (perfluoro-octanoic acid) and perfluoro-decanoic acid, were introduced to this list, owing to their dissimilarity in their structures to the known peroxisome proliferators, as a new class of peroxisome proliferators [16]. Our previous study [17] showed that PFOA markedly induced peroxisomal β -oxidation, microsomal 1-acylglycerophosphocholine acyltransferase and cytosolic long-chain-acyl-CoA hydrolase in male rat liver and that these inductions were strongly dependent on gonadal hormones. This strong sex-related difference has not been observed for other peroxisome proliferators. These findings infer that properties of this compound are very different from other peroxisome proliferators. In this context, despite the fact that PFOA was originally developed for industrial purposes, it is of interest biochemically to study its ability to increase the activity of stearoyl-CoA desaturase and its influence on the acyl composition of phospholipids. In the present work we studied the enhancing effects of PFOA on hepatic stearoyl-CoA desaturase and its influence on the acyl composition of phospholipid in male and female rats and compared them with those of typical peroxisome proliferators, clofibric acid and tiadenol.

EXPERIMENTAL

Materials

Stearoyl-CoA, clofibric acid, cytochrome *c* and bovine serum albumin were purchased from Sigma Chemical

Abbreviations used: clofibric acid, *p*-chlorophenoxyisobutyric acid; tiadenol, 2,2'-(decamethylenedithio)diethanol; PFOA, perfluoro-octanoic acid.

Co. (St. Louis, MO, U.S.A.); tiadenol and PFOA were from Aldrich Chemical Co. (Milwaukee, WI, U.S.A.); NADH was from Oriental Yeast Co. (Tokyo, Japan); testosterone propionate was from Tokyo Kasei Kogyo (Tokyo, Japan). Other chemicals were of analytical grade.

Animals and preparation of microsomes (microsomal fractions)

Male and female rats of the Wistar strain, weighing 120–150 g and 100–130 g respectively, were used. Rats were fed on a commercial diet or on that diet supplemented with 0.5% (w/w) clofibric acid, 0.5% (w/w) tiadenol or 0.02% (w/w) PFOA for 1 week. For the dose–response experiments, male rats were fed on a diet containing PFOA at doses of 0, 0.0025, 0.005, 0.01 or 0.02% (w/w) for 2 weeks. Female rats were fed on a diet containing PFOA at doses of 0, 0.01, 0.02, 0.04 and 0.08% (w/w) for 2 weeks.

Some male rats (weighing 70–80 g) were castrated 28 days before being killed. These rats were subcutaneously administered testosterone propionate (10 mg/kg of body weight) with corn oil as a vehicle once every 2 days for 3 weeks before being killed. Non-treated controls were given corn oil (0.1 ml/100 g of body weight). In some experiments, female rats (weighing 80–90 g) were treated with testosterone propionate (10 mg/kg of body weight) once every 2 days for 3 weeks before being killed. These rats were fed on a control diet or a diet containing 0.01% (w/w) PFOA for 2 weeks before being killed.

At the end of the treatments, rats were killed by decapitation. Livers were isolated and perfused with ice-cold 0.9% NaCl. The livers were homogenized in 0.25 M-sucrose and microsomes were prepared by differential centrifugation as described previously [17]. Protein concentrations in microsomal suspensions were determined by the method of Lowry *et al.* [18], with bovine serum albumin as standard.

Enzyme assays

Stearoyl-CoA desaturase was assayed essentially as described by Oshino *et al.* [19] as described previously [11] and the activity presented as the rate constant (k^+) for stearoyl-CoA-stimulated reoxidation of NADH-reduced cytochrome b_5 [20]. NADH:cytochrome c reductase and NADH:ferricyanide reductase were assayed by the methods of Oshino *et al.* [21] and Rogers & Strittmatter [22] respectively. The microsomal content of cytochrome b_5 was measured by the method of Omura & Sato [23].

Lipid analysis

Lipids were extracted from liver homogenates or microsomal suspensions by the method of Bligh & Dyer [24]. Phosphatidylcholine and phosphatidylethanolamine were isolated by t.l.c. as described by Skipski *et al.* [25] and were recovered from silica gel as described previously [12]. Fatty acids in these phospholipids were trans-methylated with BF_3 /methanol [26]. Analyses of fatty acids were performed by g.l.c. as described previously [11].

Statistical analysis

Statistical comparisons were performed by Student's t test for two means.

RESULTS

Effects of peroxisome proliferators on desaturation and acyl composition

Table 1 compares effects of peroxisome proliferators on the microsomal stearoyl-CoA desaturation system in livers of male and female rats. Treatment of male rats with clofibric acid, tiadenol or PFOA increased markedly the activity of terminal desaturase without changing considerably either the microsomal content of cytochrome b_5 or the activity of NADH:ferricyanide reductase. The activity of NADH:cytochrome c reductase was decreased effectively by the challenges with the peroxisome proliferators. PFOA increased the activity of stearoyl-CoA desaturase rather less than did the other peroxisome proliferators. Administration of clofibric acid or tiadenol to female rats increased greatly the activity of stearoyl-CoA desaturase, with changes in the other components of the microsomal desaturation system similar to those seen in male rats, although the extent of the increase was slightly less pronounced than that observed in male rats. PFOA did not increase the activity of stearoyl-CoA desaturase in the liver of female rats.

Table 2 shows effects of peroxisome proliferators on the acyl composition of hepatic phosphatidylcholine of male and female rats. The administration of the three peroxisome proliferators to male rats caused a marked increase in proportions of $\text{C}_{18:1}$, $\text{C}_{20:3, n-9}$ and $\text{C}_{20:3, n-6}$ fatty acids; the treatments decreased markedly proportions of $\text{C}_{18:2}$ and $\text{C}_{22:6}$ fatty acids. Similarly, treatment of female rats with clofibric acid or tiadenol increased the proportions of $\text{C}_{18:1}$, $\text{C}_{20:3, n-9}$ and $\text{C}_{20:3, n-6}$ fatty acids and decreased the proportion of $\text{C}_{18:2}$ fatty acid. No discernible change in the proportion of $\text{C}_{22:6}$ fatty acid was observed in female rats. Administration of PFOA to female rats did not cause a significant change in the acyl composition of phosphatidylcholine. Table 3 shows effects of the three peroxisome proliferators on the acyl composition of the hepatic phosphatidylethanolamine of male and female rats. Compared with the changes brought about in phosphatidylcholine, similar, but less pronounced, changes were observed in the proportions of $\text{C}_{18:1}$, $\text{C}_{18:2}$, $\text{C}_{20:3, n-9}$, $\text{C}_{20:3, n-6}$ and $\text{C}_{22:6}$ fatty acids in the phosphatidylethanolamine of male rats. In contrast with the situation in phosphatidylcholine, however, the treatments of rats with tiadenol or PFOA significantly increased the proportion of $\text{C}_{20:4}$ fatty acid in phosphatidylethanolamine. On the other hand, clofibric acid and tiadenol caused the same alterations in the acyl composition of the phosphatidylethanolamine of female rats as were seen in male rats, but to a less pronounced extent. PFOA did not cause any change in the acyl composition of phosphatidylethanolamine in female rats.

Dose–response effect of PFOA

Various doses of PFOA were administered to male and female rats for 2 weeks, and its influence on stearoyl-CoA desaturation was examined (Fig. 1). The treatments of male rats with various doses of PFOA caused an increase in stearoyl-CoA desaturase activity in a dose-dependent fashion (Fig. 1a). No significant change was observed in either the microsomal content of cytochrome b_5 or the activity of NADH:ferricyanide reductase (Fig. 1b). Such treatment of male rats did, however, decrease the activity of NADH:cytochrome c reductase (Fig. 1b).

Table 1. Effects of peroxisome proliferators on the hepatic stearoyl-CoA desaturation system of male and female rats

Rats were fed on a diet containing 0.5% (w/w) clofibrilic acid, 0.5% (w/w) tiadenol or 0.02% (w/w) PFOA for 1 week. The values are means $\pm$ S.D. The values in parentheses represent the numbers of animals used. * $P < 0.05$ relative to control; ^b $P < 0.01$ relative to control; ^c $P < 0.001$ relative to control. ' k^+ ' refers to the rate constant for the re-oxidation of NADH-reduced cytochrome b_5 , which was measured with stearoyl-CoA (k) and without stearoyl-CoA (k^-); the rate constant for stearoyl-CoA desaturase is given by: $k^+ = k - k^-$.

Treatment		NADH: ferricyanide reductase ($\mu\text{mol}/\text{min}$ per mg of protein)	NADH: cytochrome c reductase ($\mu\text{mol}/\text{min}$ per mg of protein)	Cytochrome b_5 (nmol/mg of protein)	Desaturase k^+ (min^{-1})
Male rats					
Control	(5)	5.66 ± 0.45	1.39 ± 0.14	0.50 ± 0.06	1.42 ± 0.33
Clofibrilic acid	(6)	5.10 ± 0.80	0.56 ± 0.16^c	0.53 ± 0.07	5.30 ± 1.04^c
Tiadenol	(4)	5.77 ± 0.65	0.44 ± 0.05^c	0.55 ± 0.03	4.02 ± 1.23^b
PFOA	(4)	4.93 ± 0.32^a	0.40 ± 0.02^c	0.54 ± 0.04	3.07 ± 0.46^c
Female rats					
Control	(7)	6.61 ± 0.82	1.91 ± 0.23	0.47 ± 0.06	1.81 ± 0.42
Clofibrilic acid	(7)	5.57 ± 1.03	0.98 ± 0.08^c	0.56 ± 0.06^a	4.94 ± 0.94^c
Tiadenol	(6)	6.72 ± 0.81	1.25 ± 0.15^c	0.58 ± 0.05^b	4.22 ± 0.76^b
PFOA	(4)	6.33 ± 0.51	1.98 ± 0.10	0.48 ± 0.02	1.93 ± 0.44

Fig. 1. Effect of the dose of PFOA on the hepatic desaturation system of male (♂) and female (♀) rats

Rats were fed on a diet containing PFOA for 2 weeks. Results are means $\pm$ S.D. for three or seven rats. Statistically significant effects are indicated by: * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$. (a) Desaturase [k^+ (min^{-1}); defined in Table 1], male rats; (b) cytochrome b_5 (●, nmol/mg of protein), NADH:ferricyanide reductase (■, $\mu\text{mol}/\text{min}$ per mg of protein) and NADH:cytochrome c reductase (▲, $\mu\text{mol}/\text{min}$ per mg of protein), male rats; (c) desaturase (k^+ , min^{-1}), female rats; (d) cytochrome b_5 (●, nmol/mg of protein), NADH:ferricyanide reductase (■, $\mu\text{mol}/\text{min}$ per mg of protein) and NADH:cytochrome c reductase (▲, $\mu\text{mol}/\text{min}$ per mg of protein), female rats.

Table 2. Alterations by peroxisome proliferators of the acyl composition of phosphatidylcholine in the liver of male and female rats

Rats were fed on a diet containing 0.5% (w/w) clofibric acid, 0.5% (w/w) tiadenol or 0.02% (w/w) PFOA for 1 week. The values are means $\pm$ s.d. for three or four rats. ^a $P < 0.05$; ^b $P < 0.01$; ^c $P < 0.001$ relative to control.

Treatment	Fatty acid . . .	Composition (mol/100 mol)									
		C _{16:0}	C _{16:1}	C _{18:0}	C _{18:1}	C _{18:2}	C _{20:3, n-9}	C _{20:3, n-6}	C _{20:4}	C _{22:6}	
Male rats											
Control		28.9±0.6	2.6±0.9	21.4±0.4	9.0±0.2	18.2±1.2	0	0.9±0.2	15.5±0.9	3.5±0.2	
Clofibric acid		37.0±1.3 ^c	4.8±1.2	8.7±2.3 ^c	24.9±1.8 ^c	9.1±0.3 ^c	1.2±0.2 ^b	3.0±0.2 ^c	10.1±1.4 ^b	1.3±0.3 ^c	
Tiadenol		36.7±1.3 ^c	2.1±0.8	11.2±1.4 ^c	16.7±1.1 ^c	9.7±1.1 ^c	1.1±0.3 ^b	2.5±0.3 ^c	17.7±0.7	2.5±0.3 ^b	
PFOA		30.3±1.2	2.5±0.6	18.2±1.4 ^a	14.2±1.1 ^c	13.0±1.4 ^b	0.6±0.3 ^a	3.6±0.3 ^c	15.8±1.3	1.8±0.2 ^c	
Female rats											
Control		17.0±1.9	0.9±0.3	35.7±0.8	5.2±0.9	15.0±0.9	0	1.5±0.3	20.8±3.6	4.0±0.7	
Clofibric acid		29.4±3.2 ^b	3.3±1.1 ^a	19.2±3.8 ^b	15.4±1.3 ^c	9.5±0.9 ^b	0.5±0.1 ^c	3.6±0.1 ^c	16.3±1.3	2.9±0.2	
Tiadenol		25.4±2.6 ^a	1.8±1.0	26.3±9.5	9.0±1.9 ^a	7.7±2.4 ^b	0.4±0.2 ^a	2.3±0.5	22.8±1.7	4.4±0.3	
PFOA		16.9±0.7	1.1±0.3	32.4±2.6	6.6±0.5 ^a	13.3±1.3	0	2.4±0.4 ^b	22.0±2.6	5.3±1.1	

Table 3. Alterations by peroxisome proliferators of the acyl composition of phosphatidylethanolamine in the liver of male and female rats

Rats were fed on a diet containing 0.5% (w/w) clofibric acid, 0.5% (w/w) tiadenol or 0.02% (w/w) PFOA for 1 week. The values are means $\pm$ s.d. for three or four rats. ^a $P < 0.05$; ^b $P < 0.01$; ^c $P < 0.001$ relative to control.

Treatment	Fatty acid...	Composition (mol/100 mol)									
		C _{16:0}	C _{16:1}	C _{18:0}	C _{18:1}	C _{18:2}	C _{20:3, n-9}	C _{20:3, n-6}	C _{20:4}	C _{22:6}	
Male rats											
Control		25.8±1.3	0.8±0.2	22.4±0.7	5.8±0.3	9.0±0.8	0	0.8±0.04	24.5±0.6	11.0±0.3	
Clofibric acid		25.7±1.8	2.0±0.6 ^a	22.1±3.9	13.1±2.5 ^b	6.1±0.9 ^a	1.1±0.4 ^b	1.8±0.4 ^a	23.7±3.5	4.8±1.3 ^b	
Tiadenol		22.8±1.0 ^a	0.6±0.2	23.2±0.5	9.3±0.6 ^c	3.9±0.4 ^c	0.5±0.1 ^c	1.1±0.2 ^a	31.6±0.7 ^c	7.1±0.4 ^c	
PFOA		22.3±0.9 ^a	1.1±0.8	25.8±0.9 ^b	7.2±0.6 ^a	5.4±0.5 ^b	0.3±0.1 ^b	1.6±0.1 ^c	31.2±0.4 ^c	5.1±0.5 ^c	
Female rats											
Control		18.4±0.9	0.9±0.4	34.3±2.4	3.6±0.5	6.3±1.1	0	1.0±0.3	25.2±0.9	10.4±0.3	
Clofibric acid		21.3±2.2	0.7±0.2	31.2±4.0	6.0±0.6 ^b	3.8±0.5 ^a	0.3±0.1 ^a	1.4±0.1	27.8±1.4 ^a	7.5±0.7 ^b	
Tiadenol		18.6±1.5	0.8±0.5	35.0±0.6	4.9±0.3 ^a	2.2±0.4 ^b	0.2±0.1 ^a	0.8±0.04	27.5±1.4	10.1±0.8	
PFOA		19.0±0.9	0.5±0.2	37.2±4.1	3.3±0.3	4.8±0.9	0	1.1±0.2	24.1±1.7	10.1±1.6	

On the other hand, the treatment of female rats with PFOA at 0.08% increased slightly, but significantly, the activity of stearoyl-CoA desaturase, whereas no change was seen in the activity of the desaturase when female rats were treated with PFOA at doses lower than 0.04% (Fig. 1c). PFOA caused no significant change in the microsomal content of cytochrome b_5 and the activities of two reductases which participate in electron transfer to the terminal desaturase (Fig. 1d).

Fig. 2 shows the effects of PFOA at various doses on the acyl composition of microsomal phosphatidylcholine and phosphatidylethanolamine in male and female rats. In association with the increase in desaturase activity shown in Fig. 1(a), a dose-dependent increase in the proportion of $C_{18:1}$ fatty acid was observed in the phosphatidylcholine of male rats. Conversely, the proportion of $C_{18:2}$ fatty acid decreased in a dose-dependent manner. $C_{20:3, n-9}$ fatty acid appeared at the doses higher

than 0.005%, and the proportion of $C_{20:3, n-6}$ fatty acid increased considerably. The proportion of $C_{22:6}$ fatty acid also decreased significantly, although no significant change was seen in the proportion of $C_{20:4}$ fatty acid. Changes in the acyl composition of phosphatidylethanolamine were similar to those seen in that of phosphatidylcholine, with the magnitude being smaller than in that of phosphatidylcholine (Fig. 2c). In contrast with the situation regarding phosphatidylcholine, the proportion of $C_{20:4}$ fatty acid in phosphatidylethanolamine was increased slightly by treatment of male rats with PFOA. No appreciable change was observed in the acyl composition of either phosphatidylcholine or phosphatidylethanolamine of female rats, except for a slight increase in the proportion of $C_{18:1}$ fatty acids in phosphatidylcholine at 0.08% PFOA and a decrease in the proportion of $C_{18:2}$ fatty acid in phosphatidylcholine at doses of 0.04 and 0.08%. No appreciable change was seen in the

Fig. 2. Effects of PFOA on the acyl composition of microsomal phosphatidylcholine and phosphatidylethanolamine in male (♂) and female (♀) rats

Rats were fed on a diet containing PFOA for 2 weeks. Results are means $\pm$ S.D. for three or four rats. Statistically significant effects are indicated by: * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$. The values for $C_{18:2}$ and $C_{22:6}$ fatty acids at the dose of 0.05% PFOA in (a) are significantly different from the control ($P < 0.001$ for $C_{18:2}$ fatty acid and $P < 0.05$ for $C_{22:6}$ fatty acid) and the value for $C_{18:1}$ fatty acid at the dose of 0.005% PFOA in (a) is not significantly different from control. Key to fatty acids: $\circ$, $C_{18:1}$; $\bullet$, $C_{18:2}$; $\blacktriangle$, $C_{20:3, n-9}$; $\triangle$, $C_{20:3, n-6}$; $\square$, $C_{20:4}$; $\blacksquare$, $C_{22:6}$. (a) Phosphatidylcholine, male; (b) phosphatidylcholine, female; (c) phosphatidylethanolamine, male; (d) phosphatidylethanolamine, female.

Table 4. Effects of testosterone on the induction by PFOA of stearoyl-CoA desaturase in rat liver

Experimental details were as described in the text. The values are means $\pm$ s.d. The values in parentheses represent the numbers of animals used. ^a $P < 0.01$ relative to corresponding control group; ^b $P < 0.001$ relative to corresponding control group. 'k⁺' is defined in Table 1.

Treatment		PFOA	NADH: ferricyanide (μ mol/min per mg of protein)	NADH: cytochrome <i>c</i> (μ mol/min per mg of protein)	Cytochrome <i>b</i> ₅ (nmol/mg of protein)	Desaturase <i>k</i> ⁺ (min ⁻¹)
Male rats						
Castrated	(3)	—	6.01 $\pm$ 0.42	1.47 $\pm$ 0.11	0.55 $\pm$ 0.04	1.76 $\pm$ 0.39
	(4)	+	7.01 $\pm$ 1.12	1.30 $\pm$ 0.24	0.59 $\pm$ 0.09	1.68 $\pm$ 0.13
Castrated plus testosterone	(3)	—	5.57 $\pm$ 0.25	1.52 $\pm$ 0.15	0.49 $\pm$ 0.03	1.34 $\pm$ 0.43
	(5)	+	5.58 $\pm$ 0.66	0.63 $\pm$ 0.06 ^b	0.69 $\pm$ 0.07 ^a	4.92 $\pm$ 0.47 ^b
Female rats						
Non-operated plus testosterone	(3)	—	6.27 $\pm$ 0.64	1.74 $\pm$ 0.19	0.53 $\pm$ 0.04	1.30 $\pm$ 0.14
	(6)	+	5.72 $\pm$ 0.73	0.83 $\pm$ 0.32 ^a	0.63 $\pm$ 0.04 ^a	3.30 $\pm$ 0.76 ^a

proportions of C_{16:0}, C_{16:1} and C_{18:0} fatty acids in both phospholipids of either male or female rats (results not shown).

Dependence of the effects of PFOA on testosterone

Castration of male rats prevented an increase in stearoyl-CoA desaturase activity by PFOA (Table 4). Administration of PFOA to castrated rats that had been pretreated with testosterone caused a marked increase in stearoyl-CoA desaturase activity. PFOA increased greatly the activity of stearoyl-CoA desaturase in the female rats which had also been treated with testosterone. These increases in stearoyl-CoA desaturase activity were achieved without appreciable changes in the microsomal content of cytochrome *b*₅ and in the activity of NADH:ferricyanide reductase. The activity of NADH:cytochrome *c* reductase was greatly decreased, in association with the increase in stearoyl-CoA desaturase activity.

DISCUSSION

With regard to a sex-related difference in the effects of peroxisome proliferators, a number of studies have been performed [27–33]. In most of those studies, only the activities of peroxisomal enzymes and peroxisome proliferation were used as parameters to estimate the response of animals to those chemicals. However, the inductions of peroxisome proliferation and peroxisomal enzymes are merely one of many responses of animals to the challenges by peroxisome proliferators. We found previously a sex-related difference in response of rats to three peroxisome proliferators, clofibrate acid, tiadenol and PFOA, in relation to four parameters, hepatomegaly, peroxisomal β -oxidation, microsomal 1-acylglycerophosphocholine acyltransferase activity and cytosolic acyl-CoA hydrolase activity [17]; especially with regard to sex-related differences, PFOA was more potent than were the other two peroxisome proliferators.

The present study showed that a sex-related difference in the increase in stearoyl-CoA desaturase activity by clofibrate acid and tiadenol was not so obvious as those observed previously for other parameters [17]. Nevertheless, the difference seen in the response of stearoyl-CoA desaturase to PFOA between male and female rats

was marked. On the basis of the results of the dose-response study, the increase in stearoyl-CoA desaturase activity in female rats by PFOA at 0.08% corresponded roughly to that in male rats at 0.003 or 0.004% PFOA, suggesting that stearoyl-CoA desaturase of male rats responded to PFOA 20–27 times as sensitively as that of female rats. The hormonal manipulation revealed that the increase of stearoyl-CoA desaturase activity by PFOA was strongly dependent on the level of testosterone in the rats. It has been established that the stearoyl-CoA desaturation system is composed of three components, namely NADH:cytochrome *b*₅ reductase, cytochrome *b*₅ and a terminal desaturase [34–37], and that the activity of NADH:cytochrome *b*₅ reductase can be assayed by using ferricyanide as an electron acceptor [38]. When cytochrome *c* was employed as an electron acceptor, the apparent rate of electron transfer through the binary electron-transport system of NADH:cytochrome *b*₅ reductase and cytochrome *b*₅ can be measured [39]. The present study showed that the peroxisome proliferators increased the activity of stearoyl-CoA desaturation by increasing terminal desaturase activity without changing the microsomal content of cytochrome *b*₅ and the activity of NADH:cytochrome *b*₅ reductase. On the other hand, the peroxisome proliferators decreased the activity of NADH:cytochrome *c* reductase, suggesting that peroxisome proliferators interfered with electron transfer at the step from NADH:cytochrome *b*₅ reductase to cytochrome *b*₅ and/or at the step from cytochrome *b*₅ to cytochrome *c*. However, the decrease in the activity of NADH:cytochrome *c* reductase does not appear to affect the increase in activity of terminal desaturase by peroxisome proliferators. Moreover, the sex-related difference seen in the increase in the activity of stearoyl-CoA desaturation by PFOA is merely due to terminal desaturase, but not due to the other two components. Recently an inhibitory effect of perfluorodecanoic acid on hepatic stearoyl-CoA desaturase in male rats was reported [40]. That the effect of perfluorodecanoic acid is opposite to that of PFOA suggests a great difference in biochemical effects among perfluorinated carboxylic acids. Further comparative investigations may be necessary to estimate the biochemical effects of perfluorinated carboxylic acids.

The administration of peroxisome proliferators resul-

ted in the increase in proportions of $C_{18:1}$ and $C_{20:3,n-9}$ fatty acids in both phosphatidylcholine and phosphatidylethanolamine. $C_{18:1}$ fatty acid is a main product of stearoyl-CoA desaturase, and $C_{20:3,n-9}$ fatty acid is a typical product derived from $C_{18:1}$ fatty acid by further desaturation and elongation when animals are under essential-fatty-acid-deficient conditions [41]. These facts may explain why an elevated activity of stearoyl-CoA desaturase caused an increase in the proportions of $C_{18:1}$ and $C_{20:3,n-9}$ fatty acids in phosphatidylcholine and phosphatidylethanolamine. Moreover, peroxisome proliferators caused an unusual acyl composition, like that in essential-fatty-acid deficiency, despite the fact that rats were fed on a nutritionally balanced diet. The greater changes in the proportions of $C_{18:1}$ and $C_{20:3,n-9}$ fatty acids in phosphatidylcholine than in phosphatidylethanolamine may be due to an induction of 1-acylglycerophosphocholine acyltransferase by the peroxisome proliferators [17,42] and to a preferential utilization of molecular species of diacylglycerol containing monoenoic acid for the formation of phosphatidylcholine [43]. A marked increase in the proportion of $C_{18:1}$ fatty acid and a corresponding decrease in the proportion of $C_{18:2}$ fatty acid were brought about in triacylglycerol by treatment of male and female rats with clofibrate or tiadenol and of male rats with PFOA, and were comparable with those of phosphatidylcholine (Y. Kawashima, N. Uy-Nu & H. Kozuka, unpublished work). The reasons for the decreases in proportions of $C_{18:2}$ and $C_{22:6}$ fatty acids and for the increase in proportion of $C_{20:3,n-6}$ fatty acid of phospholipids by the treatment of rats with the peroxisome proliferators still remain unknown. Groener *et al.* [44] previously reported similar findings, namely that changes in the acyl composition in phosphatidylcholine and triacylglycerol after refeeding starved rats with a high-carbohydrate diet were much more marked than those in phosphatidylethanolamine; the changes which they observed in the acyl composition of the three glycerolipids are similar to those observed in the present study. Accordingly, taken together, some mechanism may operate to maintain the acyl composition of phosphatidylethanolamine even under altered physiological conditions.

Since it is thought that phosphatidylcholine and phosphatidylethanolamine are produced in the endoplasmic reticulum [45] and are distributed to other intracellular organelles [46] and that the acyl composition of phospholipids affects many membrane-associated functions [47–52], peroxisome proliferators may affect membrane-associated functions through the disturbance of the acyl composition of membrane phospholipids. Accordingly, it seems that PFOA differently affect membrane-associated functions through the marked differences in the acyl composition of phospholipid between male and female rats.

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