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EFFECTS OF LONG-TERM ADMINISTRATION OF CLOFIBRIC ACID ON STEAROYL-CoA DESATURASE, 1-ACYLGLYCEROPHOSPHORYLCHOLINE ACYLTRANSFERASE AND FATTY ACYL COMPOSITION OF MICROSOMAL PHOSPHATIDYLCHOLINE IN RAT LIVER

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Abstract—Long-term effects of *p*-chlorophenoxyisobutyric acid (clofibric acid) on inductions of stearoyl-CoA desaturase and 1-acylglycerophosphorylcholine (1-acyl-GPC) acyltransferase, and on changes in fatty acyl composition of microsomal lipid in rat liver were studied. Male rats were fed clofibric acid at a dietary concentration of 0.25% for 2 or 22 weeks. Inductions of stearoyl-CoA desaturase and 1-acyl-GPC acyltransferase lasted throughout the long-term treatment and were the same as those of either young or aged rats which were treated with clofibric acid for 2 weeks. The long-term treatment of rats with clofibric acid scarcely affected components of stearoyl-CoA desaturation system other than terminal desaturase. In accordance with the induction of stearoyl-CoA desaturase, the increase in the proportion of octadecenoic acid in hepatic lipid lasted throughout the 22-week treatment. In the case of both of the long-term treatment and the short-term treatment of rats, the increase in the proportion of octadecenoic acid in microsomal phosphatidylcholine was due to the marked increase in the proportion of octadecenoic acid in position 2, but not position 1, of phosphatidylcholine. These changes in fatty acyl composition of phosphatidylcholine were not due to the alteration of the content of phosphatidylcholine in liver.

Clofibric acid is known to be a principle of clofibrate (ethyl ester of clofibric acid) which causes a marked proliferation of peroxisomes in rat liver. It has been reported that although peroxisome proliferators, including clofibric acid, cause inductions of many enzymes in rat liver [1–3], most of these enzymes have been considered to be involved in lipid catabolism. However, recent studies demonstrated that clofibrate induced microsomal glycerophosphate acyltransferase in livers of rats and mice [4, 5]. Subsequently, we found that the peroxisome proliferators, regardless of their chemical structures, induce activities of stearoyl-CoA desaturase, palmitoyl-CoA chain elongation, 1-acylglycerophosphate acyltransferase and 1-acyl-GPC acyltransferase in hepatic microsomes of rats [6–10]. These recent findings showed clearly that peroxisome proliferators are able to induce not only the enzymes involved in lipid catabolism, but also the enzymes related to lipid biosynthesis. Moreover, we showed that these microsomal enzymes induced by peroxisome proliferators cause marked alterations in fatty acyl composition of glycerolipids in rat liver [9]. Most of these findings, however, were derived from experiments on relatively short-term administration of peroxisome proliferators to animals, which raised the questions (1) whether the inductions of stearoyl-CoA desaturase and 1-acyl-GPC acyltransferaes last concomitantly throughout the treatment of animals with clofibric acid if this drug is administered to the animals for a longer period, and (2) whether fatty acyl composition of hepatic lipid changes in parallel to the changes in the activities of stearoyl-CoA desaturase and 1-acyl-GPC acyltransferase. To address these questions, in the present work we studied the effects of long-term administration of clofibric acid on stearoyl-CoA desaturase and 1-acyl-GPC acyltransferase, and the relationship between these enzymes and fatty acyl composition, hepatic lipid and phosphatidylcholine.

MATERIALS AND METHODS

Chemicals. Stearoyl-CoA, oleoyl-CoA, palmitoyl-CoA, cytochrome *c* (from horse heart), clofibric acid, snake venom (*Crotalus adamanteus*) and bovine serum albumin were purchased from Sigma Chemical Co. (St Louis, MO); NADH from the Oriental Yeast Co. (Tokyo, Japan). All other chemical reagents were of analytical grade.

Animals. Male rats of Wistar strain were used. Rats were fed *ad libitum* a commercial diet or a diet containing 0.25% clofibric acid. Material composition of commercial diet and a preparation of a diet containing clofibric acid were the same as described previously [11]. Rats aged 5 weeks at the initiation of treatment were fed a diet containing clofibric acid for various periods up to 22 weeks. Aged-matched rats aged 25 weeks at the initiation of the treatment were fed a diet containing clofibric acid for 2 weeks. For control experiments, age-matched rats which had been fed the control diet

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were used: young control rats were 6 or 7 weeks old and aged control rats were 27 weeks old on the day they were killed. All animals were exposed to an alternating light cycle (light from 0800 to 1800) and rats were killed between 1000 and 1100.

Rats were decapitated and livers were isolated. Blood remaining in the liver was washed out with cold 0.9% NaCl and the livers were rinsed with cold 0.25 M sucrose. Part of the liver was frozen at −30° for analysis of lipids. For assays of enzyme activities and analyses of microsomal lipid, the rest of the liver was homogenized with 3 vol. 0.25 M sucrose, and microsomes were prepared by differential centrifugation as described previously [6].

Enzyme assays. The terminal desaturase (stearoyl-CoA desaturase) was assayed spectrophotometrically by the method of Oshino *et al.* [12], as described previously [6], and the activity was expressed as the rate constant (k^-) of stearoyl-CoA-stimulated reoxidation of NADH-reduced cytochrome b_5.

Microsomal NADH ferricyanide reductase and NADH cytochrome *c* reductase were assayed in the presence of 1 mM KCN as described by Rogers and Strittmater [13] and Oshino *et al.* [14], respectively. The content of cytochrome b_5 in microsomes was estimated in the presence of 1 mM KCN according to the method of Omura and Sato [15].

Lipid analyses. Lipid was extracted by the method of Bligh and Dyer [16]. Phosphatidylcholine was isolated by thin layer chromatography as described previously [9]. To analyse fatty acids in position 1 and 2 of phosphatidylcholine, the phosphatidylcholine isolated was hydrolyzed by phospholipase A_2 of snake venom according to Lands and Merkl [17]. Fatty acids were analysed by gas–liquid chromatography as methyl ester, as described previously [9]. To analyse the composition of phospholipid, phospholipids were separated by thin layer chromatography as described previously [9]. The class identification was determined by reference to standards run simultaneously. The silica gel corresponding to each lipid was scraped and transferred to a tube, and lipid phosphorus was determined by the method of Rouser *et al.* [18].

Other procedures. The protein concentration was determined by the method of Lowry *et al.* [19], with bovine serum albumin as a standard. Statistical analyses were performed using Student's *t*-test for two means.

RESULTS

Figure 1 shows the time course of effects of clofibric acid on the components of microsomal stearoyl-CoA desaturation system and on the proportion of octadecenoic acid (18:1) in hepatic lipid of rats. Stearoyl-CoA desaturation system has known to be composed of three components, NADH cytochrome b_5 reductase, cytochrome b_5 and cyanide sensitive factor (terminal desaturase) [20, 21]. By the administration of clofibric acid to rats, the activity of terminal desaturase increased gradually and reached a plateau at 3 days after the initiation of the administration. The maximum specific activity observed was about 4.3 times that of control. This high specific activity of the desaturase lasted at least 22 weeks. NADH cytochrome *c* reductase activity and cytochrome b_5 content were little affected by clofibric acid throughout the administration period. Although the treatment of rats with clofibric acid slightly increased the activity of NADH ferricyanide reductase, the extent of the increase was far smaller compared to that of terminal desaturase. The increased proportion of octadecenoic acid in hepatic lipid lasted throughout the administration period of clofibric acid, as was observed with terminal desaturase. As shown in Fig. 2, by the administration of clofibric acid, the activity of 1-acyl-GPC acyltransferase increased gradually and reached a plateau at 1 week after the initiation of administration, and the induced high specific activity lasted 22 weeks after the initiation of administration, as terminal desaturase did. The specific activity of 1-acyl-GPC acyltransferase in rat liver after the long-term administration of clofibric acid was 167.7 ± 35.0 nmol/min/mg protein when oleoyl-CoA was used as a substrate, and 183.8 ± 10.2 nmol/min/mg protein when linoleoyl-CoA was used as a substrate. These values are the same as those in rat liver after the short-term administration of clofibric acid, suggesting that the substrate specificity of 1-acyl-GPC acyltransferase is not affected by the long-term administration of clofibric acid. In contrast to 1-acyl-GPC acyltransferase, the activity of 2-acyl-GPC acyltransferase in rat liver was not changed after the long-term administration of clofibric acid. The administration of clofibric acid to age-matched aged rats for 2 weeks caused induction of both stearoyl-CoA desaturase and 1-acyl-GPC acyltransferase to almost the same extent as the long-term administration of the drug to young rats (Figs. 1(a) and 2).

The effects of long-term administration of clofibric acid on fatty acyl composition of microsomal lipid in liver were studied (Table 1). In agreement with our previous findings [9], the administration of clofibric acid to young rats for 2 weeks markedly increased the proportion of octadecenoic acid and eicosatrienoic acid (20:3), and markedly decreased the proportion of octadecadienoic acid (18:2). Similarly, the administration of clofibric acid for 22 weeks altered the fatty acyl composition of microsomal lipid as did the short-term administration to young rats, although the extent of the alterations of fatty acyl composition of the rats which had been treated for the long term were somewhat smaller than those of the rats which had been treated for the short term. To confirm that the alterations in fatty acyl composition of hepatic lipid by the long-term administration of clofibric acid is not responsible for effects of aging of rats, fatty acyl composition of liver microsomal lipid of both age-matched control rats and age-matched rats which had been treated with clofibric acid for 2 weeks were analysed. As shown in Table 1, although the proportion of octadecadienoic acid and eicosatetraenoic acid were somewhat smaller and the proportion of stearic acid (18:0) was larger in age-matched control rats compared to young control rats, the proportions of other fatty acids, especially octadecenoic acid, in age-matched control rats were almost the same as those in young control rats. These results show clearly that the changes in fatty acyl composition caused by the long-term administration of clofibric acid are not due to aging of rats.

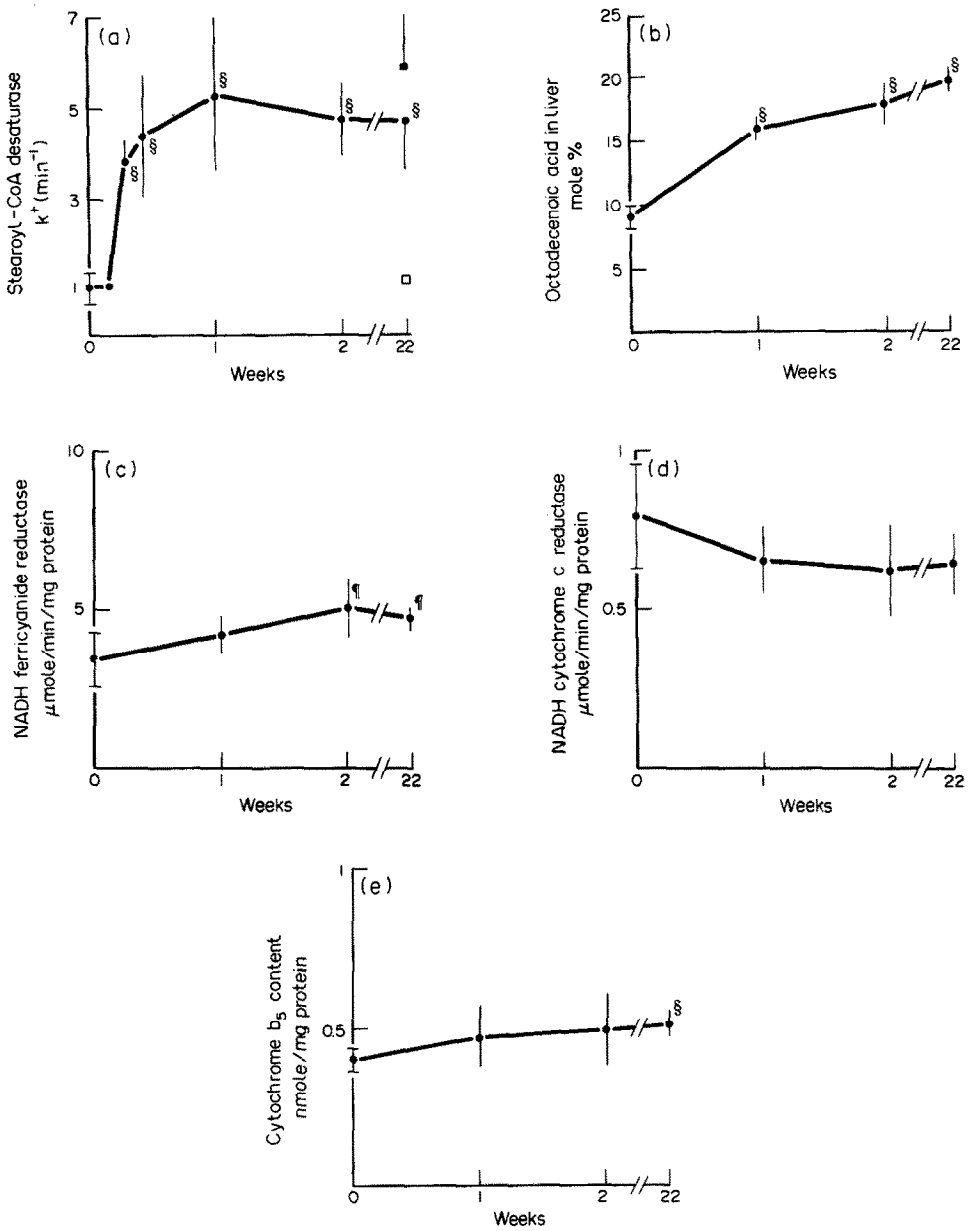

Fig. 1. Effects of long-term administration of clofibric acid on stearoyl-CoA desaturase system and proportion of octadecenoic acid in rat liver. Enzyme activities and content of cytochrome b_5 in liver microsomes were measured as described in the text. Young rats were fed a diet containing 0.25% clofibric acid for various period up to 22 weeks. Rats aged 25 weeks were fed a control diet or a diet containing 0.25% clofibric acid for 2 weeks. Values represent the mean ± SD from three to twelve animals: (a) terminal desaturase (stearoyl-CoA desaturase); (b) proportion of octadecenoic acid in hepatic lipid; (c) NADH ferricyanide reductase; (d) NADH cytochrome *c* deductase; (e) content of cytochrome b_5 in liver microsomes; (●) young rats; (□) aged rats control; (■) aged rats treated with clofibric acid for 2 weeks. § $P < 0.001$ relative to value at the initial of the treatment. ¶ $P < 0.01$ relative to value at the initial of the treatment.

Moreover, the administration of clofibric acid for 2 weeks caused similar alterations of fatty composition of microsomal lipid in age-matched rats to those in young rats.

We found [9] that the increase in the proportion of octadecenoic acid in rats liver by the short-term administration of clofibric acid was mainly due to the marked increase in the proportion of octadecenoic acid in position 2 of phosphatidyl-choline. Table 2 shows the effects of the long-term administration of clofibric acid on the fatty acyl composition of microsomal phosphatidylcholine. The changes in fatty acyl composition of phosphatidylcholine by the long-term administration of clofibric acid were simi-

Table 1. Effects of long-term administration of clofibric acid on fatty acid composition of liver microsomal lipid

Administration of clofibric acid		16:0	16:1	18:0	18:1	18:2	20:3 (N − 9)	20:3 (N − 6)	20:4	22:6
						(mole %)				
Young rats										
None	(3)	23.3 ± 2.1	2.6 ± 0.6	7.7 ± 2.5	7.3 ± 1.0	20.7 ± 2.8	0	1.4 ± 0.3	29.9 ± 1.5	7.2 ± 0.4
1 week	(3)	25.5 ± 0.9	2.1 ± 0.4	9.6 ± 1.9	14.5 ± 0.9*	9.9 ± 1.3*	2.8 ± 0.5*	4.0 ± 0.5*	28.2 ± 0.5	3.6 ± 1.1*
2 weeks	(3)	28.1 ± 1.4‡	1.9 ± 0.6	11.1 ± 2.6	16.5 ± 1.4*	8.8 ± 0.6*	3.3 ± 1.0*	4.3 ± 0.3*	22.3 ± 3.0†	3.6 ± 0.5*
22 weeks	(4)	24.9 ± 0.4	2.9 ± 0.3	13.9 ± 1.4†‖	11.3 ± 0.7*§	14.0 ± 1.4†	0.7 ± 0.3†‖	3.0 ± 0.2*§	25.6 ± 1.2†‖	3.7 ± 1.4†¶
Aged rats										
None	(4)	23.1 ± 3.0	3.3 ± 1.1	21.8 ± 3.1*	8.1 ± 0.3	15.2 ± 1.8‡	0	1.9 ± 0.3‡	20.6 ± 2.1*	6.1 ± 0.9‡
2 weeks	(4)	26.2 ± 1.0	2.8 ± 0.3	17.1 ± 3.0	13.8 ± 0.9§[bd]	10.9 ± 2.1¶[e]	1.0 ± 0.1§[a]	3.5 ± 0.3§[b]	20.7 ± 0.7[c]	4.1 ± 0.5¶

Rats were fed a control diet or a diet containing 0.25% clofibric acid for 2 or 22 weeks. Each fatty acid was indicated as a shorthand notation. The number before the colon (:) refers to the number of carbon atoms in the fatty acid chain, while the number after the colon refers to the numbers of double bonds. Each value represents the mean ± SD. Numbers in parentheses are the numbers of animals used. * $P < 0.001$ relative to young rat control; † $P < 0.01$ relative to young rat control; ‡ $P < 0.05$ relative to young rat control; § $P < 0.001$ relative to aged rat control; ‖ $P < 0.01$ relative to aged rat control; ¶ $P < 0.05$ relative to aged rat control; [a] $P < 0.01$ relative to young rat treated with clofibric acid for 2 weeks; [b] $P < 0.05$ relative to young rat treated with clofibric acid for 2 weeks; [c] $P < 0.001$ relative to young rat treated with clofibric acid for 22 weeks; [d] $P < 0.01$ relative to young rat treated with clofibric acid for 22 weeks; [e] $P < 0.05$ relative to young rat treated with clofibric acid for 22 weeks.

Table 2. Effects of long-term administration of clofibric acid on fatty acyl composition at position 1 and 2 of microsomal phosphatidylcholine in rat liver

Administration of clofibric acid		16:0	16:1	18:0	18:1	18:2	20:3 (N − 9)	20:3 (N − 6)	20:4	22:6
						(mole %)				
Total phosphatidylcholine										
None	(4)	24.3 ± 3.2	2.8 ± 1.1	25.0 ± 4.9	7.8 ± 0.7	16.7 ± 2.9	0	1.6 ± 0.3	17.5 ± 2.6	4.3 ± 0.8
2 weeks	(4)	30.3 ± 1.5‡	2.9 ± 0.3	16.0 ± 2.8‡	15.2 ± 1.8†	12.8 ± 2.7	0.8 ± 0.2†	3.8 ± 0.3†	15.8 ± 2.4	2.5 ± 0.6‡
22 weeks	(6)	29.2 ± 1.3†	2.1 ± 0.2	16.1 ± 1.0†	12.0 ± 1.8†	14.3 ± 2.2	0.4 ± 0.4	3.6 ± 0.4*	19.5 ± 1.9	2.8 ± 0.5†
Position 1 of phosphatidylcholine										
None		44.1 ± 6.6	1.6 ± 0.4	45.0 ± 8.2	7.0 ± 1.4	2.4 ± 0.5	0	0	0	0
2 weeks	(4)	57.4 ± 2.6†	3.3 ± 0.6†	30.7 ± 5.1†	6.1 ± 1.2	2.7 ± 0.7	0	0	0	0
22 weeks	(3)	56.8 ± 1.0‡	2.6 ± 0.6‡	33.9 ± 1.3	4.8 ± 0.6	1.8 ± 0.3	0	0	0	0
Position 2 of phosphatidylcholine										
None	(4)	3.0 ± 0.7	4.1 ± 1.5	0.6 ± 0.1	9.2 ± 0.8	31.0 ± 5.5	0	3.4 ± 0.8	41.1 ± 5.8	7.8 ± 1.7
2 weeks	(4)	1.5 ± 0.3†	2.6 ± 0.5	0.3 ± 0.1†	24.9 ± 2.4*	22.8 ± 4.7	1.8 ± 0.5*	7.2 ± 0.4*	34.6 ± 0.4	4.3 ± 1.3‡
22 weeks	(6)	4.5 ± 0.7‡	1.9 ± 0.5‡	2.5 ± 1.1‡	18.2 ± 3.1*	24.0 ± 3.8‡	0.8 ± 0.8	6.4 ± 0.5*	36.6 ± 3.7	5.3 ± 1.2‡

Aged rats were fed a control diet or a diet containing 0.25% clofibric acid for 2 weeks, and young rats were fed a diet containing 0.25% clofibric acid for 22 weeks. Phosphatidylcholine was isolated from microsomal lipid and then hydrolyzed by phospholipase A_2 of snake venom as described in the text. Each value represents the mean ± SD. Numbers in parentheses are the numbers of animals used.

* $P < 0.001$ relative to aged rat control. † $P < 0.01$ relative to aged rat control. ‡ $P < 0.05$ relative to aged rat control.

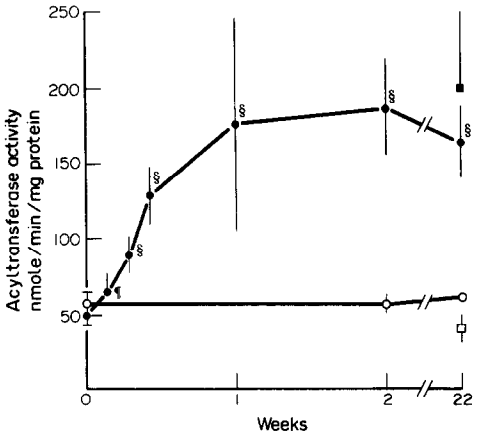

Fig. 2. Effects of long-term administration of clofibric acid on the activities of 1-acyl-GPC acyltransferase and 2-acyl-GPC acyltransferase. Young rats were fed a diet containing 0.25% clofibric acid for various periods up to 22 weeks. Rats aged 25 weeks were fed a control diet or diet containing 0.25% clofibric acid for 2 weeks. Enzyme activities in liver microsomes were assayed as described in the text. Values represent the mean ± SD from three to twelve animals: (●) 1-acyl-GPC acyltransferase activities of young rats; (○) 2-acyl-GPC acyltransferase activities of young rats; (□) 1-acyl-GPC acyltransferase activities of aged rat control; (■) 1-acyl-GPC acyltransferase activities of aged rats treated with clofibric acid for 2 weeks. § $P < 0.001$ relative to the value at the initial of the treatment. ¶ $P < 0.01$ relative to the value at the initial of the treatment.

lar to those by the short-term administration of clofibric acid to age-matched rats. To examine the effects of clofibric acid on fatty acyl composition in position 1 and 2 of phosphatidylcholine, the phospholipid was hydrolyzed by phospholipase A_2, and fatty acyl composition in the two positions was analysed separately. As shown in Table 2, the long-term administration of clofibric acid caused marked changes in the proportion of octadecenoic acid, octadecadienoic acid and eicosatrienoic acid in position 2 of phosphatidylcholine, as was observed in the short-term administration of clofibric acid to rats. On the other hand, either the long-term treatment or the short-term treatment with clofibric acid did not cause considerable changes in fatty acyl composition in position 1 of phosphatidylcholine.

Table 3 shows the effects of clofibric acid on the content of microsomal phospholipid in rat liver. Microsomal protein content per g liver was scarcely affected by either the short or long-term administration of clofibric acid so that the content of phospholipid was indicated as nmol of phosphorus/mg microsomal protein in Table 3. Although the short-term administration of clofibric acid to young rats caused a very slight increase in the content of total phospholipid due to a slight increase in the content of phosphatidylethanolamine, the content of phosphatidylcholine was scarcely altered by either the short or long-term administration of clofibric acid to rats.

DISCUSSION

The present study showed that the continuous feeding of clofibric acid for 22 weeks maintains the induced high activities both stearoyl-CoA desaturase and 1-acyl-GPC acyltransferase in rat liver concomitantly during the treatment; the specific activities increased by the long-term treatment were the same as those of either young rats or aged rats which were administered clofibric acid for 2 weeks. These facts suggest that the inductions of both stearoyl-CoA desaturase and 1-acyl-GPC acyltransferase are essential responses of rats to this drug, but not the brief response which brings about in only the first stage of the continuous intake of this drug. Many biochemical markers which respond to peroxisome proliferators to change their activities have been reported [1–10], although most of them are peroxisomal enzymes. Moreover, little information is available about long-term effects of peroxisome proliferators on the biochemical markers [11, 22]. We observed previously [11] that the extent of increase in the activity of peroxisomal β-oxidation by 22 weeks administration of clofibric acid to rats is about half that of rats treated with clofibric acid for 2 weeks. However, our previous study [8] showed that substrate specificity of 1-acyl-GPC acyltransferase induced by a short-term treatment of rats with clofibric acid is the same as that of control rats. Moreover, our recent study provided the evidence that the long-term administration of clofibric acid does not change this property of 1-acyl-GPC acyl-

Table 3. Effects of long-term administration of clofibric acid on content and composition of microsomal phospholipid in rat liver

Administration of clofibric acid		Sphingomyelin	Phosphatidylcholine	Phosphatidylserine plus phosphatidylinositol (nmole/mg protein)	Phosphatidylethanolamine	Total phospholipid
None	(6)	13.8 ± 6.4	312.9 ± 38.7	75.2 ± 17.0	116.8 ± 18.9	518.6 ± 55.9
2 weeks	(6)	13.8 ± 9.6	343.3 ± 28.2	85.6 ± 4.6	143.1 ± 14.0*	585.7 ± 45.7*
22 weeks	(6)	4.4 ± 4.6	287.2 ± 18.1	69.2 ± 3.9	114.1 ± 10.5	474.9 ± 34.1

Young rats were fed a control diet or a diet containing 0.25% clofibric acid for 2 or 22 weeks. Phospholipids were measured as described in the text. Each value represents the mean ± SD. Numbers in parentheses are the numbers of animals used.

* $P < 0.05$ relative to young rat control.

transferase, suggesting that the induced 1-acyl-GPC acyltransferase by the long-term treatment with clofibric acid is the same enzyme as that of control rats. In contrast to peroxisomal β-oxidation and stearoyl-CoA desaturase, the activity of 1-acyl-GPC acyltransferase is not changed by alterations of nutritional states, hormonal states or other xenobiotics than peroxisome proliferators. Accordingly, 1-acyl-GPC acyltransferase may be a good biochemical marker capable of responding to an intake of peroxisome proliferators.

The present study showed that the increase in the proportion of octadecenoic acid in hepatic lipid lasts throughout the long-term administration of clofibric acid to rats, as the induced activity of stearoyl-CoA desaturase did. It is well known that, by alterations in nutritional or hormonal states of rats, the fatty acyl composition (especially the proportion of octadecenoic acid) in hepatic lipid changes in association with the changes in the activity of stearoyl-CoA desaturase [23–28]. The present results, together with these findings, may strongly support an idea that stearoyl-CoA desaturase is an important factor in the regulation of proportion of octadecenoic acid in hepatic lipid. As anticipated from the results that the increased activity of 1-acyl-GPC acyltransferase lasted throughout the long-term treatment, the proportion of octadecenoic acid in position 2, but not position 1, of phosphatidylcholine was high in liver of the rats which fed clofibric acid for 22 weeks. In the previous study [29], we examined the relationship among stearoyl-CoA desaturase, 1-acyl-GPC acyltransferase and the proportion of octadecenoic acid in position 2 of phosphatidylcholine by using rats in several different kinds of physiological states (starved, starved–refed, diabetic, insulin-treated diabetic, and clofibric acid-fed rats) and reached the conclusion that changes in the proportion of octadecenoic acid in position 2 of phosphatidylcholine are related simply to the changes in activity of stearoyl-CoA desaturase except for clofibric acid-fed rats, whereas both stearoyl-CoA desaturase and 1-acyl-GPC acyltransferase may play an important role in concert to regulate the proportion of octadecenoic acid in position 2 of phosphatidylcholine in clofibric acid-fed rats. The results obtained from the present study on the long-term administration of clofibric acid may further support our previous hypothesis that the increase in the activity of 1-acyl-GPC acyltransferase, together with stearoyl-CoA desaturase, may regulate fatty acyl composition of phosphatidylcholine. It should be noted here that the administration of clofibric acid did not affect the content of phosphatidylcholine in hepatic microsomes, suggesting that the increase in the proportion of octadecenoic acid in microsomal phosphatidylcholine is not due to the increase in the content of microsomal phosphatidylcholine.

Finally, although stearoyl-CoA desaturase can be induced by changing nutritional states or hormonal states, it is impossible to maintain the activity high for even a few weeks by the manipulations of animals. Moreover, xenobiotics other than peroxisome proliferators have not been known to induce stearoyl-CoA desaturase. It is well known that changing nutritional or hormonal states of animal does not affect 1-acyl-GPC acyltranasferase. Xenobiotics which affect 1-acyl-GPC acyltransferase have not been found, except for peroxisome proliferators, so far. Therefore, our present results, clofibric acid maintaining the induced high activities of these enzymes for very long periods, may provide a useful model to study the mechanism by which peroxisome proliferators alter molecular species of phosphatidylcholine in liver of animals.

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