Role of Stearoyl-CoA Desaturase in the Modification of Acyl Composition of Hepatic Phosphatidylcholine by Peroxisome Proliferators

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The relative contribution of stearoyl-CoA desaturase and 1-acylglycerophosphocholine (1-acyl-GPC) acyltransferase, which are induced by peroxisome proliferators, to the increase in the proportion of oleic acid in C-2 position of phosphatidylcholine (PtdCho) in the liver of rats was investigated. Rats were administered either 4-chlorophenoxyisobutyric acid (clofibric acid), 2,2'-(decamethylenedithio)-di-ethanol (tiadenol) or perfluorooctanoic acid in varying doses. With administration of these peroxisome proliferators, the proportion of oleic acid in C-2 position of PtdCho in hepatic microsomes was increased in commensurate with the increase in activities of stearoyl-CoA desaturase and 1-acyl-GPC acyltransferase. Multiple regression analysis revealed that although there is a high correlation among the three parameters examined, the partial correlation between the proportion of oleic acid in the C-2 position and the activity of stearoyl-CoA desaturase is significantly higher than that between the proportion of oleic acid in the C-2 position and the activity of 1-acyl-GPC acyltransferase. The contribution of stearoyl-CoA desaturase to the increase in the proportion of oleic acid in the C-2 position thus appears greater than that of 1-acyl-GPC acyltransferase.

Key words phosphatidylcholine; oleic acid; stearoyl-CoA desaturase; 1-acylglycerophosphocholine acyltransferase; peroxisome proliferator; rat liver

Biomembranes contain a large amount of phospholipids which play physiological roles such as components of lipid bilayer, substrates for second messengers and activators for some enzymes. In cells of mammals, phospholipids show an asymmetry in the distribution of fatty acid and an individual phospholipid maintains a peculiar acyl composition. Many enzymes are involved in achieving and maintaining the acyl composition. Acyl moiety in C-2 position of phosphatidylcholine (PtdCho) is believed to be introduced by two distinct steps during its biosynthesis. One is the reaction carried out by 1-acylglycerophosphate acyltransferase and the other is the reacylation of 1-acylglycerophosphocholine (1-acyl-GPC) by 1-acyl-GPC acyltransferase. Oleic acid, which is supplied by stearoyl-CoA desaturase, is thought to be introduced to the C-2 position of PtdCho by 1-acylglycerophosphate acyltransferase or 1-acyl-GPC acyltransferase.

The administration of clofibric acid, a peroxisome proliferator, to the rat was shown to increase selectively the proportion of oleic acid in C-2 position of hepatic PtdCho, although oleic acid is known to be distributed evenly to both C-1 and C-2 positions of PtdCho in the liver of rats under physiologically normal conditions.¹⁾ This elevation by clofibric acid was demonstrated to be achieved by the induction of both stearoyl-CoA desaturase and 1-acyl-GPC acyltransferase.²⁾ Subsequently, peroxisome proliferators, unrelated structurally to clofibric acid, were shown to induce the two enzymes and to cause selective increase in the proportion of oleic acid in C-2 position of hepatic PtdCho.^{3,4)} These findings stimulated our interest regarding the relative importance of the two enzymes as a determinant for the increase in proportion by peroxisome proliferators of oleic acid in C-2 position of PtdCho. To address this question, rats in the present study were administered three different types of peroxisome proliferators with varying doses and the effect

of these proliferators on the relationship among the proportion of oleic acid in C-2 position of PtdCho, the activity of stearoyl-CoA desaturase and the activity of 1-acyl-GPC acyltransferase in the liver was investigated.

MATERIALS AND METHODS

Materials Palmitoyl-CoA, stearoyl-CoA, oleoyl-CoA, clofibric acid, snake venom (*Crotalus admanteus*) and bovine serum albumin were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.); NAD, NADH and CoA were from Oriental Yeast Co. (Tokyo, Japan); 2,2'-(decamethylenedithio)-di-ethanol (tiadenol) and perfluorooctanoic acid were from Aldrich (Milwaukee, WI, U.S.A.); 1-acyl-GPC (from egg PtdCho) was from Avanti Polar Lipid (Pelham, AL, U.S.A.).

Animals Male Wistar rats, weighing 120–150 g, were obtained from SLC (Hamamatsu, Japan). Rats were fed *ad libitum* on a standard diet or a diet containing clofibric acid, tiadenol or perfluorooctanoic acid for 2 weeks. The diet was admixed with clofibric acid at concentrations of 0.031, 0.0625, 0.125 or 0.25%, with tiadenol at concentrations of 0.031, 0.0625 or 0.125% and with perfluorooctanoic acid at concentrations of 0.00125, 0.0025, 0.005, 0.01 or 0.02%.

At the end of the treatments, the animals were killed by decapitation. Livers were isolated and perfused with ice-cold 0.9% NaCl. The liver was homogenized in 9 volumes of 0.25 M sucrose/1 mM EDTA/10 mM Tris-HCl (pH 7.4). Microsomes were prepared by differential centrifugation as described previously.⁵⁾ Concentrations of protein were determined by the method of Lowry *et al.*⁶⁾ with bovine serum albumin as a standard.

Enzyme Assays Stearoyl-CoA desaturase was assayed by employing microsomes as an enzyme source according to the method of Oshino *et al.*⁷⁾ as described⁵⁾ and

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the activity is presented as the rate constant (k^+) for stearoyl-CoA-stimulated-reoxidation of NADH-reduced cytochrome b_5 . Microsomal 1-acyl-GPC acyltransferase was assayed with oleoyl-CoA and 1-acyl-GPC as substrates by the method of Lands and Hart⁸⁾ as described.⁹⁾ Peroxisomal β -oxidation was assayed as the activity of cyanide-insensitive palmitoyl-CoA oxidation by the method of Lazarow and de Duve¹⁰⁾ using homogenates as an enzyme source.

Lipid Analyses Lipid was extracted from microsomal suspension by the method of Bligh and Dyer.¹¹⁾ PtdCho was isolated by TLC on silica gel G plates (Merck, Darmstadt, Germany) as described by Houlb and Skeaff¹²⁾ and was recovered from silica gel as described.¹³⁾ To analyze the acyl composition in C-2 position, PtdCho was hydrolyzed by snake venom according to Lands and Merkl.¹⁴⁾ Free fatty acid released from PtdCho was isolated by TLC on silica gel G plates using a solvent system comprised of hexane-diethyl ether-acetic acid (80:30:1, v/v). A spot of free fatty acid was detected after spraying 0.001% (w/v) primuline in acetone-water (4:1, v/v). The area corresponding to the free fatty acid was scraped off the plate and extracted as described.¹³⁾ Free fatty acid was methylated with BF_3 -methanol and the methyl esters of the fatty acids were analyzed by GLC as described.¹³⁾

Statistical Analyses Linear or multiple regression analyses were performed to evaluate the correlation between two or three parameters.¹⁵⁾

RESULTS

Male rats were fed a diet containing one of three different types of peroxisome proliferators, clofibric acid, tiadenol or perfluorooctanoic acid, for 2 weeks. The dietary concentrations of the chemicals were varied from the minimum doses required to induce significant peroxi-

somal β -oxidation to the doses which induce maximum activity. The maximum activity induced by the three peroxisome proliferators was approximately 50 nmol/min/mg protein, regardless of the type of chemical administered (Table 1).

In response to challenges by the three peroxisome proliferators, both stearoyl-CoA desaturase and 1-acyl-GPC acyltransferase in hepatic microsomes were induced in a dose dependent manner, as was also seen with peroxisomal β -oxidation (Table 1). To evaluate the effects of changes in the activities of stearoyl-CoA desaturase and 1-acyl-GPC acyltransferase on the proportion of oleic acid in C-2 position of PtdCho, PtdCho was isolated from microsomes and was hydrolyzed by phospholipase A_2 , and the fatty acids liberated were analyzed by GLC. The proportion of oleic acid in C-2 position of PtdCho was increased in a dose dependent manner, up to 3.6 times over control, by peroxisome proliferators (Table 1).

Linear regression analyses were carried out to examine the relationship between the proportion of oleic acid in C-2 position of PtdCho and the activity of either stearoyl-CoA desaturase or 1-acyl-GPC acyltransferase. One regression line was obtained for 13 sets of data between the proportion of oleic acid in the C-2 position and the activity of stearoyl-CoA desaturase, with a high correlation being seen between the two parameters ($r=0.933$, $p<0.001$) (Fig. 1). By contrast, two regression lines were obtained between the proportion of oleic acid in the C-2 position and the activity of 1-acyl-GPC acyltransferase, one for 5 sets of data from control and clofibric acid-treated rats, and the other for 9 sets of data from control, tiadenol-treated and perfluorooctanoic acid-treated rats (Fig. 2). A high correlation was confirmed for each group of data; the correlation coefficients for the group of clofibric acid and the group of tiadenol plus perfluorooctanoic acid were 0.993 ($p<0.001$) and 0.978 ($p<0.001$), respectively.

Table 1. Effects of Peroxisome Proliferators on the Proportion of Oleic Acid in C-2 Position of PtdCho, Stearoyl-CoA Desaturase and 1-Acyl-GPC Acyltransferase in Microsomes of Rat Liver

Treatment	Oleic acid in C-2 position of PtdCho (mol%)	Stearoyl-CoA desaturase (nmol/min/mg protein)	1-Acyl-GPC acyltransferase (nmol/min/mg protein)	Peroxisomal β -oxidation
Control	9.79 $\pm$ 0.82	1.38 $\pm$ 0.25	55.4 $\pm$ 6.2	4.30 $\pm$ 0.64
Clofibric acid				
0.031%	11.78 $\pm$ 1.40	1.47 $\pm$ 0.26	95.1 $\pm$ 13.8	6.18 $\pm$ 1.89
0.0625	17.85 $\pm$ 2.52	3.34 $\pm$ 0.09	137.3 $\pm$ 14.7	16.70 $\pm$ 5.32
0.125	33.36 $\pm$ 1.28	5.31 $\pm$ 0.26	237.6 $\pm$ 24.2	30.90 $\pm$ 5.03
0.25	35.41 $\pm$ 2.14	6.13 $\pm$ 0.85	253.0 $\pm$ 32.8	46.40 $\pm$ 4.59
Tiadenol				
0.031%	14.93 $\pm$ 1.73	2.79 $\pm$ 0.43	127.5 $\pm$ 12.9	14.30 $\pm$ 2.17
0.0625	19.33 $\pm$ 1.34	4.35 $\pm$ 0.80	186.7 $\pm$ 30.2	34.70 $\pm$ 2.83
0.125	25.41 $\pm$ 2.10	5.00 $\pm$ 0.52	254.8 $\pm$ 19.5	51.10 $\pm$ 5.10
Perfluorooctanoic acid				
0.00125%	11.62 $\pm$ 1.08	1.28 $\pm$ 0.12	113.9 $\pm$ 22.75	11.06 $\pm$ 0.67
0.0025	14.41 $\pm$ 1.13	1.41 $\pm$ 0.39	133.7 $\pm$ 21.89	20.60 $\pm$ 3.37
0.005	18.20 $\pm$ 2.67	2.18 $\pm$ 0.38	169.4 $\pm$ 33.31	28.40 $\pm$ 3.43
0.01	20.48 $\pm$ 0.67	3.06 $\pm$ 0.29	202.0 $\pm$ 12.28	37.20 $\pm$ 1.80
0.02	23.07 $\pm$ 2.05	3.64 $\pm$ 0.32	262.3 $\pm$ 15.03	47.00 $\pm$ 4.02

Rats were fed on a control diet or a diet containing one of the peroxisome proliferators for 2 weeks. Each value represents mean $\pm$ S.D. of four or six animals.

Fig. 1. Correlation between the Activity of Microsomal Stearoyl-CoA Desaturase and the Proportion of Oleic Acid in C-2 Position of PtdCho in Liver

Regression analysis was performed on the thirteen mean data from Table 1. The activity of stearoyl-CoA desaturase versus the proportion of oleic acid in C-2 position of PtdCho, $Y=5.258+4.530X$, $r=0.933$, $p<0.001$. $\circ$, control; $\bullet$, clofibric acid; $\blacksquare$, tiadenol; $\blacktriangle$, perfluorooctanoic acid.

Fig. 2. Correlation between the Activity of Microsomal 1-Acyl-GPC Acyltransferase and the Proportion of Oleic acid in C-2 Position of PtdCho in Liver

Regression analysis was performed on mean data from Table 1. The activity of 1-acyl-GPC acyltransferase versus the proportion of oleic acid in C-2 position of PtdCho; line A (for clofibric acid-treated), $Y = 0.249 + 0.137X$, $r = 0.993$, $p < 0.001$; line B (for tiadenol-treated plus perfluorooctanoic acid-treated), $Y = 4.80 + 0.076X$, $r = 0.978$, $p < 0.001$. ○, control; ●, clofibric acid; ■, tiadenol; ▲, perfluorooctanoic acid.

The relative contribution of stearoyl-CoA desaturase and 1-acyl-GPC acyltransferase to the increase in the proportion of oleic acid in C-2 position of PtdCho was determined by a multiple linear regression analysis. The 13 sets of data from Table 1 revealed a significant correlation among the three parameters ($Y = 2.874 + 3.176X_1 + 0.039X_2$; Y , proportion of oleic acid in C-2 position; X_1 , activity of stearoyl-CoA desaturase; X_2 , activity of 1-acyl-GPC acyltransferase; $r = 0.949$, $p < 0.001$). The partial correlation coefficient between the proportion of oleic acid and the activity of desaturase was 0.745 ($p < 0.01$), while the partial correlation coefficient between the proportion of oleic acid and the activity of 1-acyl-GPC acyltransferase was 0.491, and the partial correlation was not significant ($p > 0.05$).

DISCUSSION

The previous study showed that clofibric acid markedly induced both stearoyl-CoA desaturase and 1-acyl-GPC acyltransferase in the liver of rats.^{5,8,16} In a subsequent study, we sought to understand the role of the induced 1-acyl-GPC acyltransferase in the elevation of the proportion of oleic acid in C-2 position of PtdCho by comparing the proportion of oleic acid in C-2 position with the activities of stearoyl-CoA desaturase and 1-acyl-GPC acyltransferase in the rats of six physiologically different states: control, starved, starved-refed, diabetic, diabetic-insulin-treated and clofibric acid-treated rats.²⁾ These physiologically altered states gave distinct levels of activity of stearoyl-CoA desaturase, whereas the treatment with clofibric acid alone induced 1-acyl-GPC acyltransferase. Among the physiologically different states, the proportion of oleic acid in C-2 position of PtdCho in the rats treated with clofibric acid was unexpectedly higher than that anticipated from the level of activity of stearoyl-CoA desaturase in rats of the five

other states. We therefore postulated a distinctive role of 1-acyl-GPC acyltransferase induced by clofibric acid participating in the channeling of oleic acid to the C-2 position.²⁾ This kind of approach, however, did not give any information about the relative importance of the two enzymes, stearoyl-CoA desaturase and 1-acyl-GPC acyltransferase, as the determinant for the increase in the proportion of oleic acid in the C-2 position. Namely, to understand the relative contribution of stearoyl-CoA desaturase and 1-acyl-GPC acyltransferase to the proportion of oleic acid in C-2 position of PtdCho, the activities of both enzymes must be manipulated to produce various activity levels.

The present study showed that the treatments of rats with three different types of peroxisome proliferators increased in a dose dependent manner the proportion of oleic acid in C-2 position of PtdCho in association with the rise in activity of stearoyl-CoA desaturase and 1-acyl-GPC acyltransferase in the liver. These results made it possible to compare statistically the relative contribution of stearoyl-CoA desaturase and 1-acyl-GPC acyltransferase to the increase in the proportion of oleic acid in the C-2 position. The linear regression analysis provided a single regression line when the activity of stearoyl-CoA desaturase was compared with the proportion of oleic acid in C-2 position of PtdCho. Contrary to our expectations, however, the analysis revealed two regression lines between the proportion of oleic acid and the activity of 1-acyl-GPC acyltransferase. Moreover, upon multiple regression analysis for the relationship among the proportion of oleic acid in the C-2 position and the two enzymes, the partial correlation coefficient of 1-acyl-GPC acyltransferase was much lower than that of stearoyl-CoA desaturase. These results may indicate that the contribution of stearoyl-CoA desaturase to the increase in the proportion of oleic acid in the C-2 position is greater than that of 1-acyl-GPC acyltransferase in the liver of rats treated with peroxisome proliferators. These results, together with the previous findings,²⁾ suggest that although 1-acyl-GPC acyltransferase determines the channeling of oleic acid to C-2 position of PtdCho, the proportion of oleic acid in this position is regulated by the amounts of oleoyl-CoA which is supplied by stearoyl-CoA desaturase in hepatocytes under the influence of peroxisome proliferators.

The administration of peroxisome proliferators to rats has been demonstrated to markedly increase the hepatic content of palmitoyl-oleoyl PtdCho,^{16,17)} which is the most major molecular species of phospholipid containing unsaturated fatty acid for hepatocytes to produce from the components of self origin. Although the physiological significance of the increase in the molecular species of palmitoyl-oleoyl PtdCho by peroxisome proliferators in the liver has not been fully clarified yet, hepatocytes might produce the molecular species of PtdCho to supply rapidly a large amount of phospholipid containing unsaturated acyl moiety and to proliferate biomembranes of organelles under the influence of peroxisome proliferators.

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