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CHANGES IN LIPID METABOLIZING ENZYMES OF HEPATIC SUBCELLULAR FRACTIONS FROM RATS TREATED WITH TIADENOL AND CLOFIBRATE

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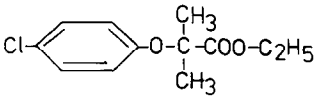
Abstract—The levels of hepatic lipid metabolizing enzymes including palmitoyl-CoA hydrolase, palmitoyl-L-carnitine hydrolase as well as some other enzymes were studied in the 100,000 *g* × 1 hr sediment, the corresponding supernatant and lipid-rich floating layer from rats fed tiadenol or clofibrate-containing diets (0.3 per cent w/w). Tiadenol administration resulted in a large increase of the total activity of palmitoyl-CoA hydrolase, and of peroxisomal-CoA oxidation, while only a moderate enhancement was obtained after clofibrate feeding. The total activity of palmitoyl-L-carnitine hydrolase was increased more by tiadenol than by clofibrate. The specific activity of the two former enzymes was decreased in the particulate MLP-fraction (100,000 *g* × 1 hr sediment containing mitochondria, peroxisomes and microsomes) after treatment with tiadenol. The specific activity of palmitoyl-CoA hydrolase was increased more than 10-fold in the cytosolic fraction after administration of tiadenol. Tiadenol increased the specific activity of palmitoyl-L-carnitine hydrolase considerably in the cytosolic fraction, but the activity of this enzyme was little affected by clofibrate treatment. The specific activity of palmitoyl-CoA hydrolase and palmitoyl-L-carnitine hydrolase increased in the lipid-rich floating layer. Since there was also a shift in the distribution of peroxisomal palmitoyl-CoA oxidation and catalase, but not of urate oxidase after treatment with the drugs, it is suggested that the drugs induce peroxisomes with altered membrane characteristics.

INTRODUCTION

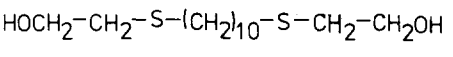
Clofibrate, (ethyl-*p*-chlorophenoxy-isobutyrate) and an alternative hypolipidemic drug, tiadenol (bis(hydroxy-ethylthio)-1, 10-decane), cause a marked proliferation of peroxisomes and mitochondria in the liver [1–10]. A number of fatty acid metabolizing enzymes localized in these organelles are also induced by clofibrate [11–17].

Other enzymes not directly involved in fatty acid metabolism, are less affected. For instance, minor changes have been observed in the activities of urate oxidase and catalase after administration of hypolipidemic drugs [5, 6, 12, 17].

Long-chain acyl-CoA plays a dominant role in governing fatty acid oxidation in mitochondria [7, 12], peroxisomes [3, 4], and in triglyceride synthesis. An alternative metabolic pathway for long-chain acyl-CoA esters is their hydrolysis to free fatty acids and CoA. A long-chain carnitine ester hydrolase has been described in rat liver [18]. The physiological function of this enzyme has not been established. Subcellular fractionation studies have shown that the palmitoyl-L-carnitine hydrolase activity is present in the microsomal fraction, whereas palmitoyl-CoA hydrolase is found in both the microsomal and mitochondrial fractions [18].



Clofibrate



Tiadenol

Fig. 1. Structural formulas of clofibrate and tiadenol.

As recently reported, the level of acyl-CoA hydrolase activity [18] and palmitoyl-L-carnitine hydrolase activity increases after clofibrate feeding (R. K. Berge, S. Skrede and M. Farsted, personal communication). The palmitoyl-L-carnitine hydrolase is localized in the microsomal fraction also after clofibrate feeding while the palmitoyl-CoA hydrolase level increases in the particale-free supernatant and decreases in the microsomal fraction. This shift of the palmitoyl-CoA hydrolase acitivity is most pronounced when the increase in liver weight is large.

The aim of the present study was to establish whether palmitoyl-CoA hydrolase and palmitoyl-L-carnitine hydrolase activities and other lipid metabolizing enzymes are altered or distributed differently in rat liver after feeding diets containing equal concentrations (0.3 per cent w/w) of clofibrate or tiadenol. The structural formulas of the drugs are shown in Fig. 1.

MATERIALS AND METHODS

Animal experiments. Male Wistar rats weighing 190–200 g were randomly selected for clofibrate or tiadenol treatment and for control experiments. The control animals were given a commercial pelleted rat food containing 55 per cent carbohydrate, 25 per cent protein, 2.1 per cent fat (w/w) and all necessary minerals and vitamins. Clofibrate or tiadenol was added by soaking the food (2 kg) with an acetone

Table 1. Effect of clofibrate and tiadenol on liver weight and protein contents*

	Control	Clofibrate	Tiadenol
Liver weight (g)	11.1–11.7	12.0–12.6	17.8–19.2
Liver/body weight (per cent)	5.2–5.5	5.9–6.2	8.6–9.1
Protein (mg/g liver)	187.4–193.4	203.8–226.5	235.0–261.7

* The value of individual rats in each treatment group are given.

solution (1.7 l.) of either drug (6 g). The organic solvent was then evaporated in a stream of hot air leaving pellets containing 0.3 per cent (w/w) of clofibrate or tiadenol. The animals were fed the drug-containing diets for ten days and then killed by decapitation. The livers were removed and immediately chilled on ice and weighed.

Preparation of fractions. The livers were homogenized in ice cold sucrose solution (0.25 M sucrose and 10 mM Hepes buffer, pH 7.4) using a Potter-Elvehjem homogenizer at 720 rpm and with two strokes of a loose-fitting teflon pestle. The following differential centrifugation fractions were collected: the precipitate from 1200 $g \times 5$ min (the nuclear fraction); the precipitate between 1200 $g \times 5$ min and 100,000 $g \times 1$ hr (the MLP-fraction consisting of mitochondria, peroxisomes and microsomes); the supernatant after 100,000 $g \times 1$ hr (the cytosolic fraction), and the floating layer formed on the top of the 100,000 $g \times 1$ hr supernatant.

Enzyme assays and analytical methods. Palmitoyl-CoA hydrolysis was measured spectrophotometrically as described previously [20]. Palmitoyl-L-carnitine hydrolase was assayed by measuring the formation of ^{14}C-palmitate [18]. Fatty acid synthetase was measured as ketoreductase, a partial reaction of the multienzyme complex [21]. Catalase [22], urate oxidase [23], and lactate dehydrogenase [25], and peroxisomal palmitoyl-CoA oxidation [3, 17] were measured by recommended procedures. The incubation medium for the latter assay consisted of: 50 mM Hepes buffer pH 7.4, 4 mM dithiothreitol, 1 mM CoA, 0.2 mM NAD^+ and 1 mM KCN, and the palmitoyl-CoA concentration was in the range of 5–15 μM. The reduction of NAD^+ was measured with an Aminco DW 2 spectrophotometer operating in the dual wavelength mode with a wavelength pair of 340 and 400 nm. All other spectrophotometric measurements were performed with a Shimadzu recording spectrophotometer model MPS 5000. Radioactivity was counted in a Packard Tricarb Liquid Scintillation Spectrometer Model 3385. Protein was determined using the Bio-Rad protein assay kit (Bio-Rad Lab., U.S.A.).

Chemicals. [1-^{14}C]palmitoyl-L-carnitine was purchased from New England Nuclear (Boston, MA). Palmitoyl-CoA, *N*-acetyl-*S*-acetacetylcysteamine, and Hepes were obtained from Sigma Chemical Co. (St. Louis, MO). The other reagents used are specified in an earlier report [20].

RESULTS

Growth was normal in all diet groups. Both clofibrate and tiadenol induced hepatomegaly (Table 1). The increase in liver weight induced by tiadenol, was much greater than that obtained in the animals treated with clofibrate. Liver protein increased after administration of either drug (Table 1). Almost 5-fold and more than 10-fold increases in total peroxisomal palmitoyl-CoA oxidation were obtained with clofibrate and tiadenol, respectively (Table 2). Total catalase activity increased a little after clofibrate whereas tiadenol treatment resulted in values 3–4 fold those of the controls. The total urate oxidase activity was not appreciably affected, whereas the

Table 2. Effect of clofibrate and tiadenol on the total activity of liver enzymes (nmol/min/g liver)*

Parameter	Control	Clofibrate	Tiadenol
Palmitoyl-CoA hydrolase	2800–3700	5500–7300	23,000–27,000
Palmitoyl-L-carnitine hydrolase	340–420	550–610	860–1000
Keto-reductase†	50–58	70–75	380–450
Peroxisomal palmitoyl-CoA-oxidation	240–254	1000–1200	2800–3400
Catalase 10^6	33–44	49–60	110–121
Urate oxidase	601–790	830–930	720–850
Lactate dehydrogenase 10^9	303–410	1000–1390	720–770
NADPH-cytochrome *c* reductase	635–660	650–780	1200–1385

* The total activity of individual rats was measured in the combined cytoplasmic extract (1200 $g \times 5$ min) and nuclear fraction.

† Measured in the cytoplasmic extract only.

Table 3. Effect of clofibrate and tiadenol on the activity of liver enzymes in the MLP-fraction, the cytosolic fraction, and the floating lipid-rich layer of the 100,000 *g* supernatant (nmol/mg protein/min)

	MLP-Fraction		
Fraction	Control	Clofibrate	Tiadenol
Palmitoyl-CoA hydrolase	51–66	55–52	44–47
Palmitoyl-L-carnitine hydrolase	4.0–4.9	5.5–5.9	2.7–3.0
Ketoreductase	N.D.*	N.D.*	N.D.*
Peroxisomal palmitoyl-CoA oxidation	1.1–1.5	5.2–6.0	10.1–11.0
Catalase $\cdot 10^3$	342–490	386–522	342–370
Urate oxidase	8.5–12.4	9.3–10.0	4.9–5.1
NADPH-cytochrome *c* reductase	20.2–25.9	18.3–19.4	8.9–9.2

	Cytosolic fraction		
Fraction	Control	Clofibrate	Tiadenol
Palmitoyl-CoA hydrolase	9–13	37–53	159–175
Palmitoyl-L-carnitine hydrolase	0.2–0.3	0.3–0.5	0.9–1.1
Ketoreductase	1.0–1.5	5.0–5.5	11.1–12.1
Peroxisomal palmitoyl-CoA oxidation	0.8–1.0	3.8–4.4	9.8–10.5
Catalase $\cdot 10^3$	217–259	313–556	875–998
Urate oxidase	N.D.*	N.D.*	N.D.*
NADPH-cytochrome *c* reductase	1.0–1.5	0.2–0.4	0.2–0.3
Lactate dehydrogenase	4400–4900	8500–9000	10,000–12,000

	Floating layer		
Fraction	Control	Clofibrate	Tiadenol
Palmitoyl-CoA hydrolase	25–28	70–80	260–275
Palmitoyl-L-carnitine hydrolase	0.3–0.4	0.5–0.6	2.1–2.5
Ketoreductase	8.5–9.8	7.4–9.6	10.5–12.5
Peroxisomal palmitoyl-CoA oxidation	1.3–1.4	2.5–3.0	9.5–10.4
Catalase $\cdot 10^3$	270–285	750–770	900–1040
Urate oxidase	N.D.*	N.D.*	N.D.*
NADPH-cytochrome *c* reductase	ND.*	N.D.*	N.D.*
Lactate dehydrogenase	6200–6500	10,000–12,000	12,500–13,500

* N.D. = not detectable.

NADPH-cytochrome *c* reductase activity was increased appreciably after tiadenol treatment and remained unchanged in the livers from the clofibrate treated animals. The lactate dehydrogenase activity was enhanced in both drug groups.

Next to peroxisomal palmitoyl-CoA oxidation, the most conspicuous alteration was observed in the activities of palmitoyl-CoA hydrolase and ketoreductase (Table 2). Again, tiadenol caused a greater increase than clofibrate. The palmitoyl-L-carnitine hydrolase activity was increased appreciably after tiadenol.

As also found in an earlier study with clofibrate (R. K. Berge, S. Skrede and M. Farstad, personal communication), drug administration resulted in little change in palmitoyl-CoA hydrolase activity in the particulate fraction and a considerable increase in the cytosolic fraction (Table 3). However, the redis-

tribution of palmitoyl-CoA hydrolase acitivity induced by tiadenol was more marked than that observed after clofibrate. Thus the specific activity in the cytosolic extract increased more than 10-fold in the tiadenol treated rats.

Palmitoyl-L-carnitine hydrolase is mainly localized in the MLP-fraction both in normal rats and after clofibrate administration. By contrast tiadenol decreased the specific activity of palmitoyl-L-carnitine hydrolase in the MLP-fraction whereas the activity increased markedly in the cytosolic fraction. The specific activity of urate oxidase in the MLP-fraction was also decreased by tiadenol, but no activity was found in the cytosolic fraction after treatment with this drug. The specific activity of NADPH-cytochrome *c* reductase in the MLP-fraction was decreased after administration of drugs, and low activity was found in the cytosolic fraction.

The specific activity of catalase was unchanged in the MLP-fraction and increased in the cytosolic fraction after drug treatment. However, the peroxisomal palmitoyl-CoA oxidation increased both in the MLP-fraction and in the cytosol, particularly in the tiadenol group. The marked increase in ketoreductase activity was observed in the cytosolic fraction and the lactate dehydrogenase activity was also elevated after drug treatment.

The specific activity of palmitoyl-CoA hydrolase in the floating layer was higher than that observed in the cytosol and an increase was found in both fractions after treatment with either drug (Table 3). Ketoreductase, lactate dehydrogenase and peroxisomal palmitoyl-CoA oxidation in the floating layer increased as in the cytosolic fraction. No urate oxidase activity and NADPH-cytochrome *c* reductase activity was found in the floating layer while catalase showed a higher specific activity in the latter fraction as compared to that found in the cytosol after feeding the drug-containing diets. Palmitoyl-L-carnitine hydrolase showed higher activity in the floating layer than in the cytosolic fraction from tiadenol-treated animals.

Table 4 shows the distribution of palmitoyl-CoA hydrolase and palmitoyl-L-carnitine hydrolase compared with the three peroxisomal 'marker' enzymes. The recovery of enzymes and protein were within the range of 86–108 per cent. After administration of clofibrate and tiadenol the amount as well as the relative specific activity of palmitoyl-CoA hydrolase decreased in the MLP-fraction and increased in cytosol fraction. This phenomenon was also observed with the catalase activity whereas palmitoyl-L-carnitine hydrolase was affected only by tiadenol. The relative specific activity of peroxisomal palmitoyl-CoA oxidation in the MLP-fraction and cytosol fraction was not changed by the drugs.

Furthermore, Table 4 shows that the amount and relative specific activities of palmitoyl-CoA hydrolase and peroxisomal palmitoyl-CoA oxidation were increased in the floating layer after drug treatment,

Table 4. Percentage distribution and relative specific activities of some enzymes in rat liver homogenates

		Absolute values*	Nuclear fraction	MLP fraction	Cytosol fraction	Floating-layer	Recovery (range per cent)
Palmitoyl-CoA-hydrolase	N	42	25(0.8)†	68(2.0)	13(0.5)	2 (0.7)	100–108
	C	79	24(0.8)	56(1.3)	21(1.4)	4 (1.4)	102–108
	T	414	23(0.8)	21(0.5)	40(2.2)	7 (2.3)	90– 93
Palmitoyl-L-carnitine hydrolase	N	4	25(0.7)	63(2.0)	3(0.2)	0.3(0.2)	89– 94
	C	7	24(0.8)	59(1.8)	4(0.2)	0.4(0.2)	86– 93
	T	17	20(0.8)	55(1.3)	7(0.2)	2.0(0.6)	86– 92
Peroxisomal palmitoyl CoA oxidation	N	3	28(0.8)	50(1.3)	17(0.7)	1 (0.4)	94– 98
	C	15	30(1.0)	48(1.2)	19(0.8)	2 (0.7)	95–102
	T	65	36(1.3)	41(1.2)	21(0.8)	3 (1.1)	96–103
Catalase	N	48	24(0.8)	46(1.5)	29(1.1)	3 (1.1)	101–104
	C	67	25(1.0)	36(0.8)	37(1.8)	4 (1.2)	99–104
	T	174	26(1.2)	27(0.6)	46(2.1)	4 (1.2)	100–108
Urate oxidase	N	7	21(0.8)	71(2.1)	N.D.	N.D.	92–100
	C	11	23(0.8)	68(1.5)	N.D.	N.D.	90– 98
	T	19	24(0.8)	56(1.2)	N.D.	N.D.	80– 90
Protein	N	2056	31	37	27	3	91–104
	C	2655	28	43	20	3	92–103
	T	4610	30	40	22	3	90–100

* The absolute values (mean) for enzyme activities are given in $\mu mol \cdot min^{-1}$ (palmitoyl-CoA hydrolase $\cdot 10^3$, catalase $\cdot 10^4$), protein in mg. The enzyme activities and the protein contents in the fractions are expressed as percent of the total in whole homogenate (i.e. cytoplasmic extract + nuclear fraction). The relative specific activities are corrected to 100 per cent recovery.

† Percentage values and relative specific activities in parenthesis.

N = normal diet control; C = clofibrate; T = tiadenol.

the former enzyme being most affected. Increased relative amount of palmitoyl-L-carnitine hydrolase in the floating layer was seen only with tiadenol. No change was observed in relative catalase activity with either drug in the floating layer.

DISCUSSION

Clofibrate was included in the present experiments as a reference drug for the study of the novel lipid lowering drug tiadenol. Our finding of increased activities of lipid metabolizing enzymes (R. K. Berge, S. Skrede and M. Farstad, personal communication) like palmitoyl-CoA-hydrolase palmitoyl-L-carnitine hydrolase and ketoreductase, and of some subcellular marker enzymes after treatment with clofibrate is in agreement with earlier reports [1–7, 12, 16, 17].

Tiadenol has been shown to increase liver catalase and induce peroxisomal proliferation [9, 10], but its effect on a wider range of hepatic enzymes has not been described. In our experiments tiadenol was considerably more potent than clofibrate in inducing enlargement of the liver and lipid metabolizing enzymes associated with the peroxisomes and other subcellular organelles. The findings were qualitatively similar with both drugs, but some of the phenomena observed with clofibrate were more clearly discernable in the experiments with tiadenol.

The shift of the palmitoyl-CoA hydrolase from the MLP fraction to the particle-free supernatant after treatment with hypolipidemic agents (Tables 3 and 4) confirms earlier findings with clofibrate (R. K. Berge, S. Skrede and M. Farstad, personal communication). Interestingly, tiadenol had a more marked effect on the distribution of the enzyme activities. Both drugs, tiadenol in particular, increase the palmitoyl-CoA hydrolase and palmitoyl-L-carnitine hydrolase activities in the MLP-fraction and there is evidence that these enzymes are localized in the endoplasmic reticulum (data to be published). Palmitoyl-CoA hydrolase was induced to a greater extent than palmitoyl-L-carnitine hydrolase. The changed distribution pattern of palmitoyl-CoA hydrolase and palmitoyl-L-carnitine hydrolase activities after tiadenol administration is accompanied by a similar redistribution of the peroxisomal markers catalase and palmitoyl-CoA oxidation, but not of urate doses. Despite the slight increase in total activity of urate oxidase in clofibrate- and tiadenol-treated rats (Table 2), the relative specific activity (Table 4) as well as the specific activity of the enzyme (Table 3) are decreased in the particulate membrane fraction (MLP-fraction). The most likely explanation of this phenomenon is that the protein content of the MLP-fraction increases in excess of the amount of peroxisomes containing the enzyme. The increase in total NADPH-cytochrome *c* reductase activity accompanied by a decrease in specific activity after tiadenol feeding is also in keeping with this interpretation.

By contrast, the amount and specific activity of catalase was increased in the cytosol-fraction by drug administration. The distribution of the catalase activity to the cytosolic fraction could be explained by a different response of the particulate (peroxisomal) and cytosolic pools of this enzyme to tiadenol, or by increased peroxisomal fragility with more leakage of the matrix enzyme to the supernatant. On the other hand, clofibrate and tiadenol could give rise to smaller or lighter lipid-rich peroxisomes containing catalase, but poor in urate oxidase activity. The presence of catalase and the lack of urate activity in the floating layer is consistent with this latter hypothesis. The three peroxisomal 'marker' enzyme activities were increased (Table 2), but showed different specific activity, relative specific activity, and amount in the isolated fractions (Tables 3 and 4). This observation in keeping with the suggestion of Flatmark *et al.* [26] that the hypolipidemic drugs may induce heterogenous peroxisomal populations, one consisting of the small or light lipid-rich vesicles capable of oxidizing palmitoyl-CoA, but poor in catalase and urate oxidase activities, another heavier or larger, containing more catalase, and the heaviest containing urate oxidase activity.

Staubli [27] observed that in normal rat liver most of the peroxisomes or 'microbodies' formed a 'nucleotid' or core. His study provides evidence that an incomplete or aberrant type of organelle is produced after treatment with clofibrate. Some 'microbody' membranes were connected to the smooth-surfaced endoplasmic reticulum by means of hook- or finger-like extensions. It is possible that the newly induced peroxisomes are functionally different from the peroxisomes of normal rat liver containing a nucleotid or core. Therefore, the different distribution of the peroxisomal marker enzymes and the microsomal palmitoyl-CoA hydrolase after treatment with hypolipidemic drugs could be related to the origin of peroxisomes.

The study of enzyme activities in different subcellular fractions after treatment with hypolipidemic drugs may shed light on the biogenesis of the peroxisomes. A series of experiments with a number of drugs inducing intracellular organelles and more refined sedimentation procedures are in progress in our laboratory.

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