Biochemical Pharmacology, Vol. 35, No. 16, pp. 2727–2730, 1986.
Printed in Great Britain.

0006-2952/86 $3.00 + 0.00
Pergamon Journals Ltd.

DIFFERENT INDUCTION OF MICROSOMAL CARBOXYLESTERASES, PALMITOYL-CoA HYDROLASE AND ACYL-L-CARNITINE HYDROLASE IN RAT LIVER AFTER TREATMENT WITH CLOFIBRATE

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(*Received* 6 *December* 1985; *accepted* 17 *February* 1986)

Abstract—The levels of hepatic carboxylesterases, including palmitoyl-CoA hydrolase and decanoyl-D,L-carnitine hydrolase, were studied in total homogenates and subcellular fractions prepared from the livers of male rats fed diets containing 0.3% clofibrate. The microsomal carboxylesterase as well as the fatty acyl-thioesterase are differently induced by clofibrate feeding. The specific activities of acetanilide carboxylesterase and decanoyl-D,L-carnitine hydrolase increased more than 3-fold in the microsomal fraction, compared to pellet-fed control animals. The microsomal activities of palmitoyl-CoA hydrolase and propanidid hydrolase were decreased by about 20 to 40% in clofibrate-treated rats. The specific clofibrate hydrolase activity remained unchanged after clofibrate administration, indicating that this microsomal carboxylesterase is not induced by its own substrate. The data suggest a different distribution of the differing carboxylesterase along the endoplasmic reticulum.

Four chemically distinct carboxylesterases exist in rat liver microsomes [1], which can be differentiated with selective substrates. The esterases with pI 6.4 and 6.2 hydrolyze propanidid and aspirin; the quantitative most important esterase with pI 6.0 cleaves clofibrate; the esterase with pI 5.6 deacylates acetanilide and lysophospholipids, and finally the esterase with pI 5.2 acts on acyl-L-carnitines, especially of medium chain length [2–5]. It has recently been shown that the drug-metabolizing carboxylesterase of pI 6.2/pI 6.4 was identical with the palmitoyl-CoA hydrolase from rat liver endoplasmic reticulum [3]. Since the apparent K_m-value observed with palmitoyl-CoA was very low, and because palmitoyl-CoA has a strong inhibitory effect on the other activities of these carboxylesterases, and finally since long-chain acyl-CoA is the most prominent natural substrate, it seems justified to speak of a palmitoyl-CoA hydrolase rather than a non-specific esterase [3, 4, 6].

Hypolipidemic drug administration, exemplified by clofibrate, induces an increase in liver palmitoyl-CoA and palmitoyl-L-carnitine hydrolase activities and peroxisomal β-oxidation [7–9]. Environmental contaminants, such as the plasticiser di-(2-ethylhexyl)phthalate, and aspirin also modulate the lipid metabolism and behave like peroxisome proliferators [10–12]. As the described compounds are structurally unrelated, the reports may provide support for the hypothesis that common induction mechanism(s) exist for peroxisome proliferation and the increase in the activities of some peroxisome-associated enzymes [13]. Thus, the results suggest that an important regulation of hepatic fatty acid metabolism by peroxisome proliferators may be attributed both to a cellular control of substrate concentration (long-chain acyl-CoA), and to organelle biogenesis [13–15].

The aim of the present study was to investigate the induction of different carboxylesterases or hydrolases in the subcellular fractions. We have observed that the drug-metabolizing carboxylesterases in rat liver can be selectively inhibited by 1 mM bis(4-nitrophenyl)-phosphate [1, 4] and can be estimated by selective xenobiotic or lipid substrates. These findings should enable us to answer the question if hypolipidemic drug-toxifying carboxylesterases are induced by their substrates, and which of the endoplasmic reticulum hydrolases will be enhanced by clofibrate feeding.

MATERIALS AND METHODS

Male Wistar rats weighing 190–200 g were fed on a commercial diet with or without addition of 0.3% (w/w) clofibrate for 10 days [8]. After decapitation the livers were removed, homogenized and subcellular fractions prepared as earlier described [15]. The hydrolysis of 2 mM clofibrate, 2 mM propanidid, and 10 mM decanoyl-D,L-carnitine was followed by autotitration with 40 mM NaOH at a constant pH of 8.0 and 37° without addition of buffer [2, 5]. The emulsion of both drugs were stabilized by sonification (150 W, 2 min; Branson sonifer B 12, Danbury, CT) with 0.01% Triton X-100. The liberation of aniline from acetanilide was assayed with the diazotation procedure described before [16]. Palmitoyl-CoA hydrolase was estimated radiochemically [7]. One unit corresponded to the release of 1 μmol acid per min. Anti-palmitoyl-CoA hydrolase serum was

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Table 1. Effect of clofibrate on the total activity of liver carboxylesterases and hydrolases

Hydrolysis of different substrates	Control	Clofibrate
Palmitoyl-CoA	*2.5 (2.4–2.7)	*5.9 (5.8–6.1)
Propanidid	*8.1 (7.9–8.4)	*7.3 (7.2–7.6)
Clofibrate	*27.2 (25.7–29.5)	*26.8 (25.8–29.7)
Acetanilide	†120 (115–128)	†215 (183–252)
Decanoyl-D,L-carnitine	†318 (280–360)	†650 (610–710)

The values are means of triplicate measurements and in the parentheses the range are given. The values are given as *(μmol/min/g liver) and †(nmol/min/g liver).

raised in rabbits as described [3]. Enzymes or cell fractions were immuno-titrated with appropriate amounts of antiserum, in constant volumes of 150 mM KCl/15 mM Hepes, pH 7.4 [3]. Inhibition studies were performed as described [3] by pre-incubation with 1 mM bis(4-nitrophenyl)phosphate in 50 mM Tris/HCl buffer pH 8.0 for 30 min at 25°.

RESULTS AND DISCUSSION

In this study we have used the substrates propanidid and palmitoyl-CoA for the enzyme pI 6.2/6.4, clofibrate for the enzyme, pI 6.0, acetanilide for the enzyme pI 5.6 and decanoyl-D,L-carnitine as substrate for the enzyme pI 5.2 to measure and discriminate for microsomal hydrolases and carboxylesterases.

Growth was normal in both diet groups and as expected clofibrate induced hepatomegaly [8]. In accordance with earlier results [8, 9, 15], the total decanoyl-D,L-carnitine hydrolase and palmitoyl-CoA hydrolase activities increased more than 2-fold after clofibrate treatment (Table 1). In contrast to the enhanced hydrolysis of palmitoyl-CoA by clofibrate administration, the total propanidid hydrolase activity (an enzyme which appears to be identical to the microsomal palmitoyl-CoA hydrolase) remained nearly unchanged [3]. The total hydrolysis of clofibrate was also unchanged by clofibrate treatment. The carboxylesterase with pI 5.6 deacylates lysophospholipids and acetanilide. Table 1 shows that this enzyme activity was increased by the peroxisome proliferator, clofibrate.

The subcellular distribution pattern of protein and some marker enzymes by the classical differential centrifugation procedure of rat liver homogenates, was essentially as described previously both in the control and clofibrate treated animals [7, 15]. Administration of the peroxisome proliferator increased the percentage of protein recovered in the M- and L-fractions, with a concomitant decrease of the microsomal protein pool. The recovery of protein and the enzyme activities was in the range 88–106%. The distribution pattern of the marker enzymes for mitochondria, lysosomes and microsomes was in general similar in both groups of animals in agreement with previous findings on the specific activities [7, 15] (data not shown). Figure 1 compares the specific activities of the measured enzymes in liver cell fractions from normal and clofibrate treated rats. In normal rats the highest specific activities of the hydrolases and carboxylesterases were found in the microsomal fraction. In this fraction, the specific activity of the clofibrate hydrolase remained nearly unchanged, indicating that this carboxylesterase was not induced by its own substrate as also found in

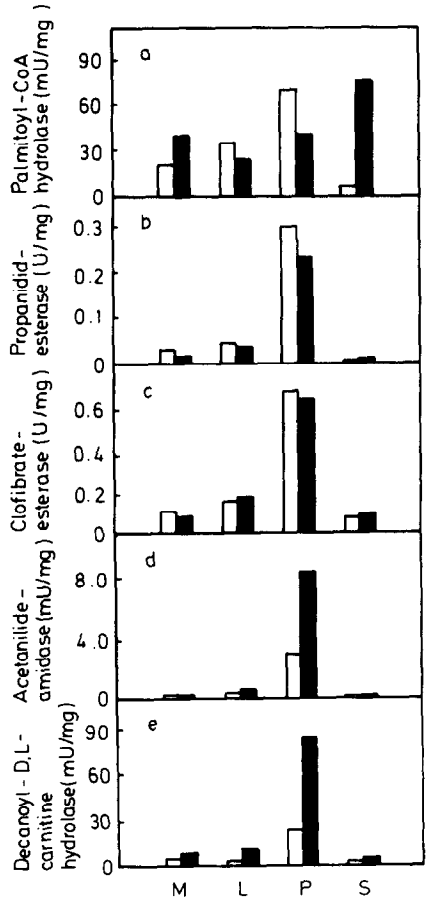

Fig. 1. Specific activities of carboxylesterase activities in subcellular fractions of normal (□) and clofibrate treated rats (■). The substrates used were: a, palmitoyl-CoA; b, propanidid; c, clofibrate; d, acetanilide; e, decanoyl-D, L-carnitine. The subcellular fractions: M, mitochondrial fraction; L, light mitochondrial fraction; P, microsomal fraction and S particle-free supernatant (cytosol). The values are means of duplicate measurements of two individual animals for normal and clofibrate treated rats each.

Table 2. Selective inhibition of palmitoyl-CoA and decanoyl-L-carnitine hydrolase activities in liver cell fractions from normal and clofibrate treated rats

		*Residual activities (%) after inhibition with 1 mM bis(4-nitrophenyl)-phosphate and anti palmitoyl-CoA hydrolase serum		
		Palmitoyl-CoA hydrolase		Decanoyl-D,L-carnitine hydrolase
Cell fractions	Animals	Organophosphate inhibition	Immunotitration	Organophosphate inhibition
Mitochondria	Normal	91 (83–96)		n.d.
	Clofibrate	102 (96–110)	87 (86–90)	n.d.
Light Mitochondria	Normal	64 (60–68)	n.d.	6 (5–8)
	Clofibrate	75 (73–77)	n.d.	10 (8–12)
Microsomes	Normal	5 (4–6)	8 (5–10)	6 (4–8)
	Clofibrate	25 (23–30)	34 (29–38)	10 (8–12)
Cytosol	Normal	92 (81–115)	86 (79–102)	n.d.
	Clofibrate	107 (104–117)	81 (70–90)	n.d.

* The values are means of triplicate measurements (tested with the cellular fractions, N = 8). The values in parenthesis are the range of triplicate measurements. n.d., Not determined.

whole homogenates (Table 1). The specific activity towards propanidid and palmitoyl-CoA in the corresponding fraction decreased after clofibrate feeding. However, the propanidid hydrolase activity decreased approximately 20% while the palmitoyl-CoA hydrolase activity was decreased about 40%. Furthermore, the enhanced activity of the palmitoyl-CoA hydrolase in the cytosol fraction after clofibrate treatment was not observed using propanidid as substrate. In contrast to the microsomal palmitoyl-CoA hydrolase and propanidid esterase activities, the specific activities of the acetanilide and decanoyl-D,L-carnitine hydrolase significantly increased (3.1-fold) in the microsomal fraction. No increased activity was observed in the cytosol after clofibrate administration. The induction is in the same order of magnitude as found for the surface area of smooth endoplasmic reticulum [17], and therefore may be interpreted as a different distribution of the esterases and hydrolases along the endoplasmic reticulum. These results are in accordance with the former reports on the induction of liver palmitoyl-L-carnitine hydrolase activity after clofibrate and tiadenol feeding [7, 8, 14] and that palmitoyl-L-carnitine hydrolase is an enzyme different from the palmitoyl-CoA hydrolase [6, 18].

Treatment of the microsomal fraction of normal rats with bis(4-nitrophenyl)phosphate as well as anti-palmitoyl-CoA antiserum (raised against purified microsomal enzyme from normal rats) gave about 90% inhibition of the palmitoyl-CoA hydrolase activity (Table 2). A similar inhibition of the organophosphate inhibitor was observed for the decanoyl-D,L-carnitine hydrolase activity. By clofibrate feeding, however, the antiserum gave about 70% inhibition of the enzyme activity. In contrast, the corresponding activities of the mitochondria and cytoplasma both in normal and clofibrate treated rats were slightly affected by treatment with this antiserum or with the organophosphate. Recently, we have shown that a long-chain acyl-CoA hydrolase activity was associated with peroxisomes [15]. Thus, the slight inhibition of the light mitochondrial fraction with this inhibitor may be explained that the peroxisome-associated palmitoyl-CoA hydrolase activity is poorly inhibited with the organophosphate inhibitor. Alternatively, the slight inhibition of the light mitochondrial fraction may be explained by contamination with endoplasmic reticulum vesicles. We have suggested that hypolipidemic drugs increase the activity of a "microsomal-like" palmitoyl-CoA hydrolase in the cytosol as there was a decrease in the microsomal hydrolase activity with a concomitant increase in the cytosol enzyme [7, 9]. From the inhibition experiments in this report the hydrolase seemed not to move out of the endoplasmic reticulum. However, the substrate specificity may be changed by clofibrate treatment and the cytosol seems to contain at least three different palmitoyl-CoA hydrolases [7].

In conclusion, we have found that carboxylesterases as well as fatty acyl-thioesterases are differently induced by clofibrate administration. Whereas the specific activity of the clofibrate esterase remain unchanged, the microsomal palmitoyl-CoA (propanidid) hydrolase activity was decreased [7]. In contrast, the two other carboxylesterases, cleaving acetanilide and acyl-carnitine, were induced 3.1-fold.

Acknowledgements—The work was supported by grants from the Deutsche Forschungsgemeinschaft, the Norwegian Research Council for Science and Humanities, the Norwegian Cancer Society and the Norwegian Society for Fighting Cancer. We also thank the Norwegian Royal Ministry for Foreign Affairs for financial support (scholarship to Dr. Mentlein).

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