EFFECTS OF CLOFIBRATE ON THE INTRACELLULAR LOCALIZATION OF PALMITOYL-CoA HYDROLASE AND PALMITOYL-L-CARNITINE HYDROLASE IN RAT LIVER

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1. Introduction

Feeding clofibrate to rats will induce a prominent increase in the number of peroxisomes [1] and of the capacity for fatty-acid oxidation [2,3].

Clofibrate feeding also results in a great increase in the hepatic content of free CoA and long-chain acyl-CoA [4–6] and an increase of the acyl-CoA hydrolase activity [7].

We now report that clofibrate treatment of rats results in an increase of the activity of both palmitoyl-L-carnitine hydrolase and palmitoyl-CoA hydrolase. Also a subcellular redistribution of the latter enzyme is found, possibly due to release from structurally changed microsomal membranes (endoplasmatic reticulum) to the particle-free supernatant.

2. Experimental procedures

2.1. *Rats and diets*

Male Wistar-Møll rats (220–340 g) were randomly selected for control and clofibrate-treated groups, and given ordinary, commercial food or the same food containing 0.3% (w/w) clofibrate as in [4]. Both groups were fed 10–14 days before they were killed.

2.2. *Preparation of subcellular fractions*

The subfractions of liver homogenates were prepared essentially as in [8] with some modifications [9].

2.3. *Ultrogel AcA-44 chromatography and determination of the app.* M_r*- values*

The particle-free supernatant of liver was applied to a column of Ultrogel AcA-44 (1.6 × 90 cm), equilibrated with 10 mM Hepes buffer (pH 7.4) and 150 mM KCl. The flow rate was 0.16 ml/min and the hydrolase was eluted with the same buffer. Palmitoyl-CoA hydrolase and decanoyl-CoA hydrolase activities were measured.

2.4. *Enzyme assays and other analytical methods*

Hydrolysis of decanoyl-CoA, palmitoyl-CoA and palmitoyl-L-carnitine was assayed as in [10,11].

The marker enzymes were: For the outer mitochondrial membrane, amine oxidase and ATP-dependent long-chain acyl-CoA synthetase (using oleate as the substrate) [12,13]; for the inner mitochondrial membrane, carnitine palmitoyltransferase [14]; for the mitochondrial matrix, glutamate dehydrogenase [15]; for the light mitochondrial fraction, acid phosphatase (lysosomes) [16] and catalase (peroxisomes) [17]; for the microsomal fraction NADPH-cytochrome *c* reductase [18]; and for the plasma membranes phosphodiesterase I [19].

Fatty acid synthetase [20] and the partial reactions of the multi-enzyme complex, ketoreductase [21], were assayed as in [20,21]. Protein was determined using the Folin-Ciocalteu reagent [22].

2.5. *Materials*

[1-^{14}C]Palmitoyl-CoA and [1-^{14}C]palmitoyl-L-carnitine were purchased from New England Nuclear, Boston, MA. Malonyl-CoA and *N*-acetyl-*S*-acetoacetyl-cysteamine were purchased from Sigma Chemical Co., St Louis, MO. Ultrogel AcA-44 was from LKB, Bromma. Other reagents were as in [4,23,24].

3. Results and discussion

Feeding clofibrate to rats resulted in an increase in wet liver weight from 11.6 + 0.2 g (n = 7) to 13.4 ± 0.2 g (n = 5), representing 5.4 ± 0.4% of the body weight in treated rats and 4.2 ± 0.6% in controls. The body weight of the rats did not change.

The subcellular distribution of palmitoyl-CoA hydrolase, palmitoyl-L-carnitine hydrolase and different marker enzymes in control and clofibrate-treated rats is shown in table 1. The distribution pattern of the marker enzymes was roughly the same in the two groups. The recovery of enzymes and the protein was in the range 80–110%.

In both groups, the protein content was evenly increased in all cellular subfractions. The marker enzymes (see section 2.4 above) for inner mitochondrial membrane [14], mitochondrial matrix [15], lysosomes [16], peroxisomes [17], microsomes [18], and plasma membrane [19] were distributed as might be expected [4,11]. The cross-contamination between fractions, calculated from the marker enzymes, was low except for the presence of marker enzymes for the mitochondrial outer membrane, peroxisomes and

Table 1
Distribution of palmitoyl-CoA hydrolase, palmitoyl-L-carnitine hydrolase and marker enzymes in subfractions of rat liver homogenates fed a normal diet *vs* fed a diet containing clofibrate

	Absolute values in homogenate	Percentage values					
		N	M	L	P	S	%
Palmitoyl-CoA hydrolase	44.7 ± 7.8 94.9 ± 19.1	14.1 ± 1.3 14.3 ± 3.0	20.6 ± 0.9 19.2 ± 2.2	6.7 ± 2.2 5.8 ± 0.4	54.3 ± 5.8 27.8 ± 2.5	15.2 ± 5.1 42.3 ± 8.4	96–110 95–109
Palmitoyl-L-carnitine hydrolase	11.5 ± 0.9 20.9 ± 4.0	13.9 ± 3.6 15.6 ± 1.3	7.2 ± 1.2 9.2 ± 0.8	3.6 ± 1.3 2.8 ± 0.9	56.4 ± 3.6 50.2 ± 0.5	13.2 ± 4.0 15.2 ± 0.5	94–103 93– 98
Long-chain acyl-CoA synthetase	40.0 ± 10.5 68.1 ± 9.7	17.6 ± 2.3 16.2 ± 1.8	21.9 ± 8.2 20.8 ± 5.3	4.8 ± 1.6 7.1 ± 2.4	38.6 ± 7.3 31.4 ± 6.5	15.6 ± 8.6 17.4 ± 7.3	80– 93 80– 92
Carnitine palmitoyltransferase	9.9 ± 3.2 28.4 ± 6.3	18.0 ± 3.1 19.4 ± 2.8	62.5 ± 4.1 61.8 ± 3.1	6.4 ± 2.1 7.1 ± 1.8	10.3 ± 3.2 7.5 ± 2.8	3.4 ± 0.7 4.0 ± 0.9	98–105 97–106
Phosphodiesterase I	261.7 ± 15.2 253.3 ± 75.4	50.2 ± 9.1 54.2 ± 7.7	13.1 ± 2.1 14.2 ± 0.8	7.0 ± 0.8 9.1 ± 4.1	24.8 ± 1.0 23.6 ± 0.8	2.1 ± 0.2 2.3 ± 0.3	96–103 97–105
Amine oxidase	9.4 ± 2.3 12.1 ± 2.1	18.3 ± 3.5 15.7 ± 4.0	52.9 ± 2.0 46.4 ± 1.0	8.8 ± 1.0 9.6 ± 0.8	11.5 ± 0.7 9.4 ± 2.1	3.7 ± 0.5 4.6 ± 0.5	95– 99 86– 96
Glutamate dehydrogenase	1265 ± 152 1834 ± 250	22.2 ± 2.3 18.5 ± 3.4	67.8 ± 2.5 61.0 ± 2.4	7.6 ± 0.5 6.3 ± 1.0	8.0 ± 3.2 2.2 ± 0.6	0.7 ± 0.4 2.0 ± 0.3	85– 99 86– 96
Acid phosphatase	85.2 ± 11.1 97.1 ± 2.3	10.6 ± 3.8 10.0 ± 3.4	26.8 ± 6.8 28.0 ± 3.3	24.1 ± 1.5 33.3 ± 1.1	30.9 ± 2.6 12.1 ± 2.4	18.3 ± 5.9 18.5 ± 1.8	98–110 96–102
Catalase	103.1 ± 2.3 153.8 ± 11.1	3.3 ± 0.8 6.1 ± 0.3	13.8 ± 1.8 17.3 ± 2.5	25.8 ± 3.2 26.0 ± 2.8	11.8 ± 3.1 8.9 ± 2.8	48.8 ± 4.0 42.9 ± 6.0	102–110 100–108
NADPH-cytochrome *c* reductase	33.2 ± 12.8 37.8 ± 12.4	9.9 ± 3.1 20.3 ± 0.7	5.0 ± 1.1 5.6 ± 0.5	6.1 ± 5.0 7.3 ± 1.2	49.4 ± 6.6 42.4 ± 5.4	23.3 ± 7.3 18.8 ± 7.2	94–103 94–102
Keto-reductase	11.3 ± 5.2 20.2 ± 3.8	0.2 ± 0.1 0.2 ± 0.1	10.1 ± 2.3 12.7 ± 3.2	2.5 ± 1.3 3.5 ± 0.8	0.2 ± 0.1 0.2 ± 0.1	83.4 ± 4.2 80.7 ± 2.8	97–106 98–107
Protein	2088 ± 225 2456 ± 300	17.4 ± 7.3 15.9 ± 3.0	20.4 ± 0.9 18.5 ± 2.2	5.4 ± 0.7 6.4 ± 0.4	20.9 ± 2.1 18.1 ± 2.1	34.4 ± 2.8 32.2 ± 1.7	96–108 92–110

The absolute values for enzyme activities are given in μmol . min^{-1}, except for carnitine palmitoyltransferase, which activity is given in arbitrary units. Protein is given in mg. The enzyme activities and the protein content in the subfractions are expressed as percent of the total in whole homogenate (i.e., cytoplasmic extract + nuclear fraction). The % values are given as the means + SD of 4 animals. The upper row for each enzyme represents normal fed, and the lower row clofibrate fed rats. For identification of the fractions, see fig.1.

microsomes in the particle-free supernatant. The distribution of amine oxidase and long-chain acyl-CoA synthetase, an enzyme probably also localized to the peroxisomes [25], might indicate some fragmentation of the outer mitochondrial membrane and the peroxisomes. The high catalase activity found in the particle-free supernatant might, however, be due to 'soluble' catalase which is higher in male rats [26].

The palmitoyl-L-carnitine hydrolase was localized in the microsomal fraction (table 1, fig.1) both in clofibrate-treated and control rats, confirming earlier findings [11,27]. Clofibrate treatment induced an increase of its specific activity, in the total homogenate of 1.9-times ($p < 0.001$) in the microsomal fraction by ~1.6-times ($p < 0.01$), but not in the other fractions.

Table 1 and fig.1 confirm the dual localization of palmitoyl-CoA hydrolase [11]. However, palmitoyl-CoA hydrolase was redistributed after clofibrate treatment (table 1, fig.1), as its activity increased in the particle-free supernatant, but decreased in the microsomal fraction. The increase in the particle-free supernatant was, however, most pronounced in rats with the greatest liver weight gain (fig.1). In clofibrate-treated rats, but not in control rats, the relative specific activities in the particle-free supernatant and in the microsomal fraction increased and decreased, respectively, in relation to the increased liver/body weight ratio (fig.2).

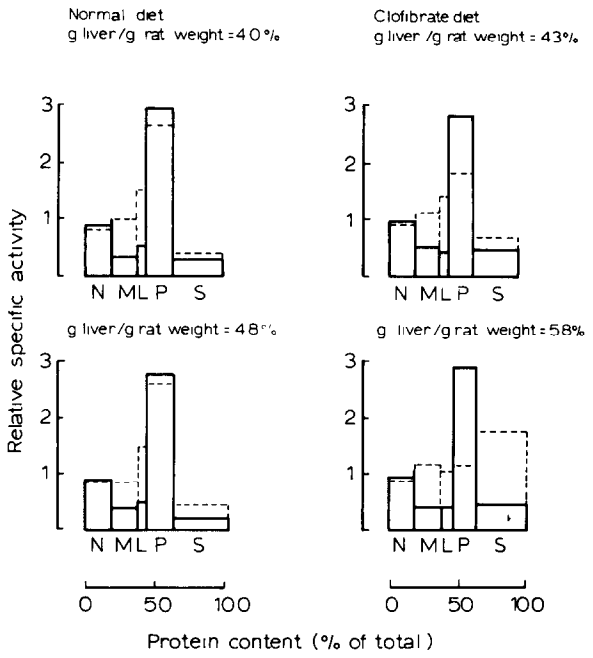

Fig.1. Distribution pattern of palmitoyl-CoA hydrolase (– – –) and palmitoyl-L-carnitine hydrolase (——) in livers from 2 normal and 2 clofibrate-treated rats related to the ratio between liver weight and body weight of the animals. The relative specific activity (% total activity/% total protein) is plotted against the relative protein content in each fraction (%): N, the nuclear fraction; M, the mitochondrial fraction; L, the light mitochondrial fraction; P, the microsomal fraction; S, the particle-free supernatant.

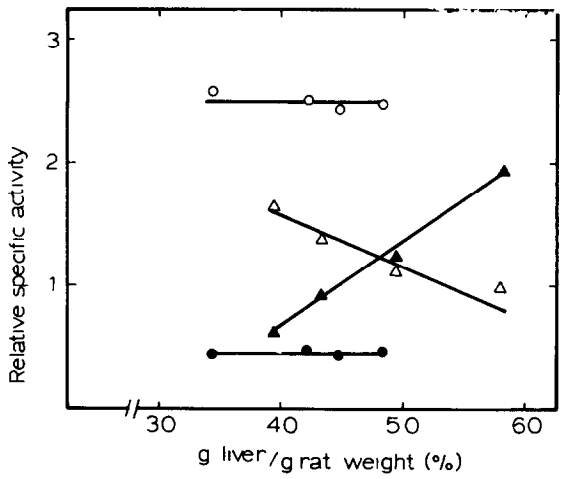

Fig.2. The relative specific activity of palmitoyl-CoA hydrolase in the microsomal fraction and the particle-free supernatant in normal and clofibrate-treated rats. The relative specific activity of palmitoyl-CoA hydrolase in microsomal fraction (open symbols) and particle-free supernatant (filled symbols) of livers of rats fed a normal diet (circles) and in clofibrate-treated rats (triangles).

The specific activity of palmitoyl-CoA hydrolase in the particle-free supernatant increased ~5-times ($p < 0.001$) after clofibrate treatment, and in the total homogenate and in the mitochondrial fraction 1.5–1.7-times ($p < 0.01$). No significant differences were found in the other fractions.

In the total homogenates, the specific activities of long-chain acyl-CoA synthetase (using oleate as substrate) and carnitine palmitoyltransferase increased ~1.5- and 2.5-times, respectively, following clofibrate treatment, in agreement with [28,29]. The specific activities of fatty acid synthetase (not shown) and ketoreductase increased 2–3-times (table 1). The increased specific activity of catalase after clofibrate treatment, is in agreement with [4]. Glutamate dehydrogenase activity was increased ~25% in clofibrate treated rats (table 1).

The increased palmitoyl-CoA hydrolysis in the particle-free supernatant (table 1, fig.1,fig.2) might be derived from microsomes, structurally changed by clofibrate. This microsomal hydrolase may be partly soluble [23] and localized in microsomal membrane fragments different from those containing NADPH-cytochrome *c* reductase which showed the same distribution in the two groups (table 1). The contribution of the mitochondrial matrix palmitoyl-CoA hydrolase activity [24] to the activity in the particle-free supernatant was probably low as the recovery of glutamate dehydrogenase was low in this fraction (table 1). Since the recovery of catalase and acid phosphatase in the particle-free supernatant was about the same in the two groups (table 1), it seems unlikely that the whole increase of palmitoyl-CoA hydrolase activity in the particle-free supernatant from clofibrate treated rats was derived from the peroxisomes, which contain acyl-CoA hydrolase activity [30]. However, some contribution might occur as the recovery in the light mitochondrial fraction was decreased (table 1) and the relative specific activity of the palmitoyl-CoA hydrolase decreased in the L-fraction (fig.1).

When particle-free supernatant, both from control and clofibrate-treated rats, was chromatographed on Ultrogel AcA-44 (see section 2.3 above), the palmitoyl-CoA hydrolase activity eluted as 3 peaks (I,II,III) in both groups (not shown). Clofibrate administration thus seemed not to induce the synthesis of a new cytosolic acyl-CoA hydrolase. Peak I coincided with the void volume and contained ketoreductase activity and probably represented the palmitoyl-CoA hydrolase activity associated with the fatty acid synthetase complex [31]. The recovery of palmitoyl-CoA hydrolase activity after gel filtration was low, 5–10%, in agreement with [32,33].

When the particle-free supernatant from the clofibrate treated rats was extracted by water-saturated butanol as in [23], and applied to the Ultrogel AcA-44 column, the palmitoyl-CoA hydrolase activity eluted mainly as peak II and III with a recovery of 80–90%. The purified microsomal palmitoyl-CoA hydrolase [23] eluated only as peak II (not shown).

In summary, the results indicate that the main increase of palmitoyl-CoA hydrolase in the particle-free supernatant after clofibrate treatment is due to drug-induced alterations of the endoplasmic reticulum. Also, the particle-free supernatant of rat liver possesed 2 or 3 different palmitoyl-CoA hydrolases, one of which (peak I) may be a part of the fatty acid synthetase complex. The enzyme found in peak II seemed to be 'microsome associated' [23,34]. The enzyme in peak III possibly is a lower M_r form of the peak-II enzyme in [32,33].

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