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EFFECT OF 3- AND 4-THIA-SUBSTITUTED FATTY ACIDS ON GLYCEROLIPID METABOLISM AND MITOCHONDRIAL β-OXIDATION IN RAT LIVER

JON SKORVE, BENTE RUYTER,* ARILD C. RUSTAN,* ERLING N. CHRISTIANSEN,* CHRISTIAN A. DREVON* and ROLF K. BERGE†

Laboratory of Clinical Biochemistry, University of Bergen, N-5021 Haukeland Sykehus; and
* Section for Dietary Research, Institute for Nutrition Research, University of Oslo, P.O. Box 1046 Blindern, N-0316 Oslo 3, Norway

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Abstract—Treatment of normolipidemic rats by alkylthiopropionic acid (CETTD), resulted in a dose- and time-dependent increase in total dihydroxyacetone phosphate acyltransferase (DHAPAT) activity, in extent comparable to that of 3-thiadicarboxylic acid (BCMTD) and alkylthioacetic acid (CMTTD). Thus, in CETTD- and CMTTD-treated rats, the specific DHAPAT activity increased in the microsomal, peroxisomal and mitochondrial fractions. In contrast, repeated administration of the peroxisome proliferator, BCMTD, decreased the specific DHAPAT activity both in the peroxisomal fraction and in purified peroxisomes. A three-fold increase in specific activity was, however, revealed in the mitochondrial fraction. Whether the variation of the DHAPAT activity in the mitochondrial and microsomal fractions among the feeding groups can be explained by increased number of enlarged and small peroxisomes sedimenting in the fractions, are to be considered. Subcellular fractionation studies confirmed previous findings that rat liver glycerophosphate acyltransferase (GPAT) was located both in mitochondria and the microsomal fraction. BCMTD was considerably more potent than CMTTD in stimulating the microsomal and mitochondrial GPAT activities. Administration of CETTD marginally affected the isoenzymes of GPAT. Diacylglycerol acyltransferase (DGAT) activity was increased by 35% in BCMTD and CMTTD treated rats, but by administration of CETTD the enzyme activity was decreased by more than 80%. The acyl-CoA cholesterol acyltransferase (ACAT) activity was marginally affected in animals treated with BCMTD, CMTTD and CETTD. Thus, the results indicate that the initial steps in the synthesis of triacylglycerols and ether glycerolipids as well as the last step in triacylglycerol synthesis could not be identified as mediating the fat accumulation or the lowering of triacylglycerol content in liver of CETTD, or BCMTD and CMTTD treated rats. On the other hand, CMTTD increased the palmitoyl-CoA oxidation in mitochondria, and CETTD considerably inhibited the activity. Therefore, it is conceivable that the development of fatty liver with CETTD is mostly due to inhibition of mitochondrial β-oxidation.

Recently, we have reported that a number of changes are observed in hepatic fatty acid metabolism and morphology when male rats are given non-β-oxidizable sulfur-substituted fatty acid analogues: 1,10-bis (carboxymethylthioldecane (3-thiadicarboxylic acid BCMTD‡) and (CMTTD) [1, 2]. The analogues are hypolipidemic, cause peroxisomal proliferation and they lower the serum concentration of both cholesterol and triacylglycerol [3]. In contrast, a β-oxidizable sulfur substituted fatty acid analogue (CETTD), only marginally affects the peroxisomal capacity to oxidize long-chain fatty acids. The most striking effect of repeated administration of CETTD is accumulation of lipids in serum as well as liver [1, 2].

The effects of BCMTD, and especially CMTTD, are consistent with the lowering of plasma triacylglycerol obtained with feeding fish oils containing long-chain w-3 fatty acids [4, 5]. To characterize the alterations taking place in hepatic lipid metabolism in the presence of fatty acid analogues we evaluated the activity of enzymes which could modify the flux of fatty acids into ether lipids and triacylglycerols and subsequently into VLDL. GPAT and DHAPAT may have considerable potential for this regulation as they catalyse the initial esterification steps in triacylglycerol synthesis. Since it has been shown in cultured rat hepatocytes that esterification of diacylglycerol and cholesterol could be inhibited by eicosapentaaenoic acid [6] it was also of interest to examine whether enzyme activities of DGAT or ACAT might contribute to the lowering of plasma triacylglycerol and cholesterol concentration.

The present study was therefore undertaken to examine some of the possible mechanisms, including mitochondrial fatty acid oxidation, via which

† To whom all correspondence should be addressed.

‡ Abbreviations: BCMTD, 1,10-bis(carboxymethylthio)decane (3-thiadicarboxylic acid); CMTTD, 1-(carboxymethylthio)tetradecane (alkylthioacetic acid); CETTD, 1-(carboxyethylthio)tetradecane (alkylthiopropionic acid); *s*, the average sedimentation coefficient for a group of particles at 4°; M, heavy mitochondrial fraction; L, light mitochondrial fraction; P, microsomal fraction; GPAT, glycerophosphate acyltransferase; DHAPAT, dihydroxyacetone phosphate acyltransferase; DGAT, acyl-CoA:1.2-diacylglycerol acyltransferase; ACAT, acyl-CoA:cholesterol acyltransferase.

BCMTD, CMTTD and CETTD may influence concentration of plasma and liver triacylglycerol and cholesterol.

MATERIALS AND METHODS

Chemical and drugs. [1-^{14}C]Palmitic acid [1-^{14}C]palmitoyl-CoA, [1-^{14}C]oleyl-CoA, L-[1-^{14}C]-carnitine, L-[1-^{14}C]palmitoyl-L-carnitine and [1-^{14}C]glycerol-3-phosphate, purchased from New England Nuclear (Boston, MA, U.S.A.), were mixed with unlabelled palmitic acid, palmitoyl-CoA, oleyl-CoA, L-carnitine and glycerol-3-phosphate (Sigma Chemical Co., MO, U.S.A.) to obtain a proper specific radioactivity. 1,10-Bis-(carboxymethylthio)decane (BCMTD, thiadicarboxylic acid), 1-(carboxymethylthio)tetradecane (CMTTD, alkylthioacetic acid) and 1-(carboxyethylthio)tetradecane (CETTD, alkylthiopropionic acid) were prepared as earlier described [3, 7]. All other chemicals were obtained from common commercial sources and were of reagent grade.

Animals and treatments. Male Wistar rats from Möllegaard Breeding Laboratory, Ejby, Denmark, weighing 170–180 g, were housed individually in metal wire cages in a room maintained at 12 hr light–dark cycles and at a constant temperature of 20 ± 3°. The animals were acclimatized for at least 1 week under these conditions before the start of the experiment. BCMTD, CMTTD and CETTD were suspended in 0.5% sodium carboxymethyl cellulose (CMS). The individual agents were administered by gastric tube feeding in a volume of 1 mL once a day and the animals were killed after 8 hr of starvation. The animals were separately treated with the fatty acid analogues and the doses were the same for all of them: BCMTD, CMTTD and CETTD 150 mg/day/kg body weight. The control animal groups received CMS instead of active compounds. All animals had free access to water and food [8].

The body weights were measured daily. At the end of the experiments, the fasted rats were lightly anesthesized and cardiac puncture was performed. Then the livers were removed and immediately chilled on ice and weighed.

Preparation of total homogenate and the different subcellular fractions. Livers from individual rats were homogenized in ice-cold sucrose-medium (0.25 M sucrose in 10 mM Hepes buffer, pH 7.4 and 2 mM EDTA), centrifuged and the resulting nuclear plus postnuclear fractions were used as the total homogenate.

For further analysis of postnuclear fractions differential centrifugation samples from three animals were pooled, and a mitochondrial-enriched fraction (M), peroxisome-enriched fraction (L), microsomal fraction (P) and cytosolic fraction (S) were isolated [9, 10]. Purified peroxisomes were prepared as described earlier [10].

The variation of the response from animal to animal was estimated separately for selected enzymes in the group of control animals.

Enzyme assays. The subcellular marker enzymes were determined as previously outlined [9, 10] and protein was assayed by a Bio-Rad protein assay kit (Bio-Rad, Richmond, CA, U.S.A.).

The enzymatic activity of glycerophosphate acyltransferase (EC 2.3.1.15), and palmitoyl-CoA dependent dehydrogenase (usually termed peroxisomal-β-oxidation) were determined in accordance with earlier description [1–3, 9, 10].

DHAPAT was determined according to Schutgens *et al.* [11], with minor modifications as described by Ruyter *et al.* (manuscript in preparation). The initial assay conditions were 75 mM sodium acetate buffer (pH 5.4), 8 mM sodium fluoride, 8 mM $MgCl_2$, 0.4 mM palmitoyl-CoA, 41.7 mg/mL bovine serum albumin (BSA), 0.10 mM [U-^{14}C]dihydroxyacetonephosphate (30,000 cpm/nmol) and 20–80 μg protein homogenate in a total volume of 0.12 mL. Incubations were carried out for 40 min at 37°.

DGAT was measured by the method of Coleman and Bell [12], slightly modified [6] using [^{14}C]oleyl-CoA and 1,2-dioleoylglycerol as substrates. Enzyme activity was assayed at 23° in 500 μL of Tris buffer (175 mM, pH 7.8) containing BSA (15 μM). After 5 min preincubation, microsomes (20 μg/mL) and 1,2-dioleoylglycerol (125 μM) dissolved in ethanol (10% v/v final concentration) were added and incubation continued for 10 min. The incubation was terminated by adding 20 volumes of chloroform/methanol (2:1, v/v). Serum (20 μL) was added to supply triacylglycerol as unlabelled carrier. After

Table 1. Liver parameters in controls and rats fed sulfur-substituted fatty acid acid analogues

Parameter measured	Control	BCMTD	CMTTD	CETTD
Peroxisomal β-oxidation (nmol/min/g liver)	450 ± 20	4500 ± 50*	1800 ± 200*	800 ± 100*
Mitochondrial β-oxidation (nmol/min/g liver)	280 ± 40	300 ± 40	380 ± 20*	90 ± 30*
Number of mitochondria per cell	3110	5460*	3460	3095
Triacylglycerol (μmol/g liver)	4.6 ± 0.4	2.5 ± 0.1*	3.7 ± 0.2*	19.5 ± 0.3*

Values from the livers represent means ± SD of nine animals in each treatment group (see Materials and Methods). The morphological data are the means of three animals.

* $P < 0.02$ for comparison between control and other groups.

extraction [13], the residual lipids were redissolved in *n*-hexane and applied on TLC plates.

ACAT was assayed using [^{14}C]oleoyl-CoA and endogenous microsomal cholesterol as substrates [14]. The incubation mixture of 500 μL contained potassium phosphate buffer (0.1 M, pH 7.4), defatted BSA (15 μM) and [^{14}C]oleoyl-CoA (0.1 μCi/mL, 30 μM). The incubation mixture was preincubated at 37° for 10 min before microsomal protein (300 μg/mL) was added and the incubation continued for 2 min. The incubation was terminated by adding 20 volumes of chloroform/methanol (2:1, v/v), and further treated as described for DGAT.

Palmitoyl-CoA and palmitoyl-L-carnitine oxidation was evaluated by measuring appearance of acid-soluble products [15].

Morphological methods. Calculations to estimate cytoplasmic volume, per cent of cytoplasma occupied by peroxisomes and mitochondria, number of organelles per unit of cytoplasmic volume and average volume of peroxisomes and mitochondria were done according to current morphometric principles.

Presentation of the results. The variation in the response from animal to animal was estimated separately for selected enzymes in total liver homogenates in the control group and treated animals (N = 3). Data on enzyme activities in total liver homogenates are presented as means ± SD. For isolation of cellular fractions the postnuclear fractions from three animals were pooled. The tabulated values on enzymatic activities in cellular fractions of treated rats are given as means. In all tables and figures the observed differences are compared to 0, 7 and 14 days, tabulated as means ± SD to day 0 (N = 12 in control group and N = 3 in the experimental group) as there were no significant changes in the control animals of the 14-day period (varied between ±5–10%). $P > 0.05$ was taken to be statistically insignificant.

RESULTS

Serum lipids

Dietary intake and body weight of all animal groups were similar. Repeated administration of BCMTD and CMTTD at doses of 150 mg/day/kg body weight for 10 days caused a reduction of serum triacylglycerols and cholesterol [3]. Treatment with CETTD in the same dose resulted in an increase of serum triacylglycerols level whereas the cholesterol concentration was unchanged, relative to control animals [3].

Hepatic pleiotropic response

The relative liver size [3], peroxisomal β-oxidation and the number of peroxisomes and mitochondria per hepatocytes increased in BCMTD-treated rats, whereas the hepatic triacylglycerol content was decreased (Table 1). Administration of CMTTD also decreased the concentration of hepatic triacylglycerol. In addition the alkylthioacetic acid increased the peroxisomal oxidation of fatty acids, accompanied with proliferation of peroxisomes, although to a much lesser extent than observed with 3-thiadicarboxylic acid. Formation of enlarged mitochondria was also observed [1–3]. The most striking

Table 2. DHAPAT and GPAT activities in homogenate and isolated cellular fractions

Enzymes	Administration	Specific activity					Relative specific activity				Percentage distribution		
		Homogenate	M-fraction	P-fraction	L-fraction	Peroxisomes	M-fraction	P-fraction	L-fraction	Peroxisomes	M-fraction	P-fraction	L-fraction
DHAPAT	Control	0.079±0.008	0.054±0.014	0.18±0.03	0.82±0.10	2.01±0.10	0.7	2.3	10.4	25.4	10–15	23–26	52–64
	BCMTD	0.146±0.018*	0.165	0.20	0.69	1.35	1.1	1.4	4.7	9.2	17–18	14–17	52–59
	CMTTD	0.38±0.009*	0.134	0.24	1.18	2.45	1.0	1.7	8.6	17.8	13–15	19–22	58–60
	CETTD	0.27±0.020*	0.084	0.21	1.23	2.60	0.7	1.7	9.7	20.5	12–16	21–25	58–62
GPAT	Control	0.76±0.10	1.21±0.18	3.48±0.33			1.6	4.6			28–33	53–57	
	BCMTD	2.96±0.11*	2.68	9.63			0.9	3.3			17–20	48–54	
	CMTTD	1.71±0.26*	1.99	7.16			1.2	4.2			22–24	56–61	
	CETTD	0.58±0.10	0.97	2.51			1.7	4.3			15–17	56–61	

The tabulated values represent the means ± SD for twelve control animals and three animals of the experimental groups at day 10 with a dose of 150 mg/day/kg body weight. Specific activity is expressed in nmol/min/mg protein and the relative specific activity is expressed as the ratio of specific activity in the cellular fractions and that in the whole homogenate. The sum of the enzyme activities are expressed as per cent of the whole homogenate.

* $P < 0.02$ for comparison between control and other groups.

effect of alkylthiopropionic acid was increased hepatic triacylglycerol content and inhibition of mitochondrial β-oxidation amounting to 70% (Table 1).

The distribution of protein and marker enzymes for mitochondria, peroxisomes and microsomes were for all groups essentially similar to our previous findings for rat liver homogenates [1, 2]. High purity of the cellular fractions, was found, as judged by the distribition of marker enzymes. Contamination of the peroxisomal fraction with mitochondria and microsomes amounts to 2–3% and 5–8%, respectively, whereas 2–4% contamination with mitochondria and peroxisomes was found in the microsomal fraction. Based on total activities, the amount of rotenone-insensitive NADPH-cytochrome *c* reductase and urate oxidase suggest a 8–10% contamination with microsomes and peroxisomes in the mitochondrial fraction [1–3].

Effect on DHAPAT activity

In homogenates of rat liver the highest specific DHAPAT activity was found in the peroxisomal fraction amounting to 52–64% of the total enzyme activity (Table 2). The peroxisomes were enriched 10-fold in the L-fraction and 25-fold in the peroxisomal pellet as compared to the homogenate, whereas only 10–15% and 23–26% of the activity was observed in the mitochondrial (M)- and microsomal (P)-fractions, respectively. Administration of the sulfur substituted fatty acid analogues increased the DHAPAT activity in total liver homogenates. Alkylthioacetic acid, and especially the potent peroxisome proliferator 3-thiadicarboxylic acid, altered the appearance of the DHAPAT activity among cellular fractions (Table 2). First, the recovery (as percentage of total activity) and the specific activity of DHAPAT in the M-fraction was significantly increased. Secondly, the enzyme was enriched only 5-fold in the L-fraction and 9-fold in the peroxisomal pellet as compared to homogenate in BCMTD-treated animals (Table 3).

The distribution of DHAPAT activity among cellular fractions in CETTD-treated rats was mostly similar to untreated control rats, except that the specific enzyme activity was increased in the mitochondrial and peroxisomal fractions (Fig. 1).

Repeated administration of the three sulfur substituted fatty acid analogues caused a dose-related increase of DHAPAT activity in the total homogenate (Table 3) and mitochondrial fraction (data not shown), which was already revealed during the first two days of treatment (Table 4, Fig. 1). Subsequently, with longer feeding periods, the enzyme activity in the BCMTD-, CMTTD- and CETTD-treated rats continued to increase up to 10 days feeding and amounted 3-fold, 2.5-fold and 2-fold, respectively in mitochondrial DHAPAT activity (Fig. 1). A lower increase of enzyme activity was observed in total liver homogenates (Table 4) and microsomal fractions (Fig. 1). The dose- (Table 3) and time- (Fig. 1) course showed that CMTTD and CETTD administration increases the DHAPAT activity in the peroxisomal fractions, whereas the enzyme activity in this fraction and purified peroxisomes was decreased in BCMTD exposed rats (Table 3, Fig. 1).

Table 3. Effects of sulphur substituted fatty acid analogues on DHAPAT and GPAT activities as a function of increasing doses

Administration	Cellular fractions	DHAPAT activity at doses						GPAT activity at doses						
		0	75	150	250	400	500	750	0	75	150	250	400	500	750
BCMTD	E	12.0±0.8	15.5±0.2	24.2±2.4	19.0±1.4		26.3±3.8		116.2±8.8	265.5±8.6	422.7±10.2	455.6±6.6		397.4±15.4	
	L	0.59±0.06	0.71	0.48	0.46		0.45								
	M								1.05±0.25	1.48	2.16	2.21		2.74	
	P								2.85±0.20	6.40	8.44	8.26		8.21	
CMTTD	E	12.5±0.7	16.8±1.9	20.1±3.3	18.6±0.9		25.1±3.2	26.6±3.8	116.8±6.7	166.7	201.9	217.5		294.5	276.1
	L	0.63±0.03	0.73	0.77	0.73		0.95	0.93							
	M								1.10±0.21	1.31	1.36	1.52		2.22	1.75
	P								3.06	3.86	5.24	5.30		7.50	6.98
CETTD	E	12.3±0.4		20.3±1.0		23.3±1.8		23.4±4.9	120.2±8.6		125.1		123.5		129.9
	L	0.61±0.06		0.87		0.87		0.91							
	M								1.06±0.18		0.90		0.61		0.82
	P								2.86±8.30		3.60		4.07		4.55

The tabulated values represent the means ± SD for twelve control animals and means of three treated animals. The activity in homogenates (E) is expressed as nmol/min/g liver whereas the activities in cellular fractions are expressed as nmol/min/mg protein.

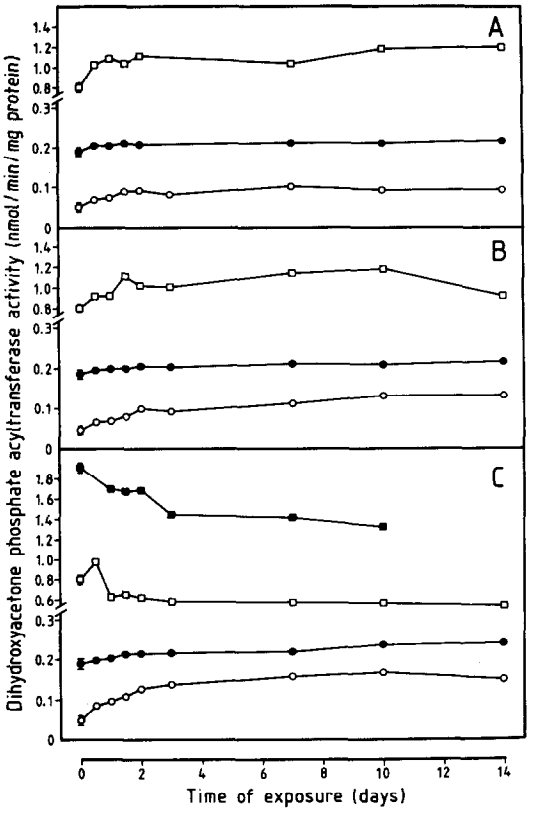

Fig. 1. Effect of CMTTD (A), CETTD (B) and BCMTD (C) exposure on dihydroxyacetone phosphate acyltransferase activity as a function of time. The enzyme activity in the mitochondrial fraction (○–○), in the peroxisomal fraction (□–□), in the microsomal fraction (●–●) and in purified peroxisomes (■–■). No significant changes were observed in the control animals of the 14-day period (5 to 10% between 0, 7 and 14 days). The tabulated values represent the means ± SD for twelve animals of three control groups and means of six animals for two feeding groups.

Effect on GPAT-activity

In homogenates of rat liver the highest specific GPAT activity was found in the microsomal and mitochondrial fraction amounting to 28–33 and 53–57%, respectively, of the total activity (Table 2). BCMTD was considerably more potent than CMTTD in causing stimulation of the GPAT activity in mitochondrial and microsomal fractions. Furthermore, maximal induction of GPAT activity occurred at different dose levels for the two inductors. CETTD feeding did not alter the GPAT activity (Table 3).

Repeated administration of BCMTD caused a dose-related increase of GPAT activity in total liver homogenates and the microsomal and mitochondrial fractions (Table 3), which was already revealed during the first day of treatment (Table 4). Administration of CMTTD also increased the GPAT activity within the first two days, whereas repeated administration of CETTD tended to decrease GPAT activity in total liver homogenates (Table 4).

Effect on DGAT activity

Microsomal DGAT activity was significantly increased (35%) in 10 days BCMTD and CMTTD treated rats. Administration of CETTD, however, reduced the DGAT activity by more than 80% (Table 5).

Effect on ACAT activity

Repeated administration of the sulfur-substituted fatty acid analogues did not significantly change the ACAT activity (Table 5) and the hepatic concentration of free cholesterol and cholesterolester (data not shown).

Effect on fatty acid oxidation

Figure 2 shows that the oxidation of [1-^{14}C]palmitoyl-L-carnitine (A) and [1-^{14}C]palmitoyl-CoA (B) in the absence of KCN, decreased as a function of time in isolated mitochondria from rats administered CETTD. After 7 days of administration

Table 4. Time-dependent changes of DHAPAT and GPAT activities in liver homogenates

Days of treatment	DHAPAT activity			GPAT activity		
	BCMTD	CMTTD	CETTD	BCMTD	CMTTD	CETTD
0		12.1 ± 1.5			116.4 ± 14.3	
0.5	13.3 ± 2.6	14.6 ± 1.2	16.5 ± 0.8*	188.1 ± 15.6*	150.6 ± 15.6*	118.6 ± 12.6
1	14.0 ± 0.8	14.0 ± 0.5	15.6 ± 1.0*	205.6 ± 8.6*	175.2 ± 10.6†	112.1 ± 10.8
1.5	19.0 ± 3.1*	14.9 ± 2.3	16.7 ± 1.4*	245.7 ± 20.1*	178.1 ± 10.6†	106.6 ± 15.1
2	18.4 ± 2.3*	16.6 ± 1.9†	20.1 ± 1.1*	280.6 ± 28.7†	162.1 ± 15.7†	95.2 ± 10.1
3	17.3 ± 3.6*	14.8 ± 1.4	15.7 ± 1.9*	310.1 ± 20.6†	205.2 ± 30.6†	101.6 ± 8.8
7	21.1 ± 1.0*	21.5 ± 1.7*	14.1 ± 3.3	421.6 ± 10.8†	290.1 ± 10.2†	90.1 ± 10.6
10	23.3 ± 2.6*	20.7 ± 1.6*	18.1 ± 4.3*	485.1 ± 6.6†	280.6 ± 26.2†	80.6 ± 6.7†
14	17.7 ± 1.1*	18.9 ± 2.9*	17.3 ± 2.9*	450.1 ± 37.8†	285.9 ± 31.4†	85.6 ± 15.2†

The tabulated values (nmol/min/g liver) represent the means ± SD of twelve control animals and three rats in each treatment group at a dose of 150 mg/day/kg body weight.

* $P < 0.01$; † $P < 0.005$. BCMTD, the thiadicarboxylic acid; CMTTD, the alkylthioacetic acid; CETTD, the alkylthiopropionic acid.

Table 5. Time-dependent changes of diacylglycerol acyltransferase (DGAT) and cholesterol acyltransferase (ACAT) in rat liver microsomes

Days of treatment	DGAT activity			ACAT activity		
	BCMTD	CMTTD	CETTD	BCMTD	CMTTD	CETTD
0		2.4 ± 0.4			1.10 ± 0.17	
0.5	2.2	2.3	3.0	0.97	1.08	1.36
1.5	2.5	2.5		1.20	1.04	1.50
2	2.3	2.1	2.7	1.01	0.99	1.24
3	2.4	2.4	2.3	0.72	0.96	1.37
7	2.6	3.2*	1.4†	1.02	0.82	1.76
10	3.2*	3.4*	0.4†	1.24	0.75	1.40

The tabulated values (nmol/min/mg protein) represent the means SD of twelve control animals (see Materials and Methods) and three rats in each treatment group at a dose of 150 mg/day/kg body weight. BCMTD, the thiadicarboxylic acid; CMTTD, the alkylthioacetic acid and CETTD, the alkylthiopropionic acid.

* $P < 0.05$, † $P < 0.01$.

a 60–80% inhibition of mitochondrial fatty acid oxidation was revealed. Notably, in the presence of KCN (Fig. 2C), the oxidation of [1-^{14}C]palmitoyl-CoA remained unaffected within 2 days of feeding. In the 3 days fed animals, however, an increased cyanide-insensitive oxidation of palmitoyl-CoA was observed, probably promoted by stimulated peroxisomal β-oxidation (Table 1).

DISCUSSION

The liver is very active in esterifying and oxidizing long-chain fatty acids. Both mitochondria and peroxisomes are capable of oxidizing fatty acids via β-oxidation. Peroxisomes appear to be of importance in oxidation of long-chain fatty acids which are found in fish oils, and dicarboxylic acids, but peroxisomes contribute overall only to a minor extent to the hepatic fatty acid oxidation.

Mitochondria, peroxisomes and endoplasmic reticulum are reported to contain enzymes that catalyse the initial steps in the synthesis of glycerolipids. The importance of peroxisomes in ether lipid biosynthesis was confirmed after demonstrating that tissues of infants without peroxisomes (i.e. cerebro-hepatorenal syndrome or Zellweger syndrome) were deficient in plasmalogen. This implies that peroxisomes play a major role in initiating ether glycerolipid synthesis.

Results from this study, confirms earlier reports of a predominant peroxisomal localization of DHAPAT in rat liver [16–18]. However, as in several other investigations, some activity was also detected in the mitochondrial and microsomal fractions, and there is still some controversy in the literature about the possibility of separate enzymes in mitochondria and/or microsomes [16–18]. The diverging effects of BCMTD, CMTTD and CETTD observed on the specific actitivies of DHAPAT in the different fractions may be taken to support the idea of separate enzymes. Thus, repeated administrations of all these compounds led to an increase in the specific activity of DHAPAT in total homogenate, in the M- and the P-fraction, while in the L-fraction and in purified peroxisomes an increase was observed in CMTTD and CETTD treated rats, and a decrease in rats treated with BCMTD.

However, the order of potency of these compounds with respect to the increase in the mitochondrial fraction (Fig. 1) was similar to that observed for peroxisomal β-oxidation and increase organelle number (BCMTD > CMTTD ⩾ CETTD) (Table 1). This increase thus seems to be coupled to the proliferation of peroxisomes. It has been shown that administration of clofibrate, a potent peroxisome proliferator led to a marked broadening in the size distribution of hepatic peroxisomes along with an increase in the average volume and diameter [19]. An increase in mean peroxisomal volume has been observed, especially in the BCMTD treated rats [3]. Consequently, it can not be ruled out that the increased specific activity of DHAPAT observed in the mitochondrial fraction may be due to an increased number of large peroxisomes sedimenting in this fraction. This slight increase in the specific activity of DHAPAT in the microsomal fraction may likewise be explained by an increased number of small peroxisomes, or it may be the result of an increased breakage of peroxisomes. Thus, treatment with peroxisome proliferators may increase the fragility of this organelle [10, 19].

Rats treated with BCMTD or CMTTD were found to decrease plasma and liver triacylglycerol levels and plasma cholesterol content, whereas CETTD treatment caused increased plasma and liver triacylglycerols [3]. The observed alterations in plasma lipids could result from either an impaired efflux of lipids from the liver, or BCMTD and CMTTD may cause triacylglycerol-lowering effect by limiting the availability of free fatty acids for synthesis of triacylglycerol. Another possibility for the fatty acid analogues to lower plasma concentrations of triacylglycerol and cholesterol might be that they interfere with triacylglycerol and cholesterol biosynthesis in the liver via an inhibition of one or more enzymes involved in these pathways.

GPAT, which is localized in mitochondria and endoplasmic reticulum (Table 2), may have regulatory potential in the triacylglycerol esterification sequence, as the contribution of peroxisomal

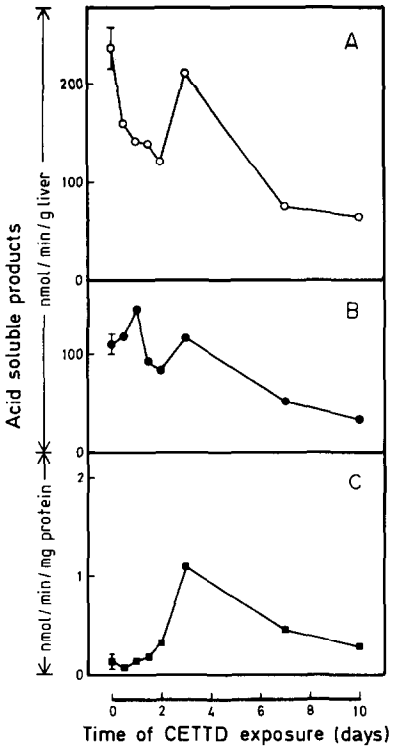

Fig. 2. Effect of CETTD exposure on [1-^{14}C]palmitoyl-L-carnithine oxidation (A) and [1-^{14}C]palmitoyl-CoA oxidation (B) in liver mitochondria in the absence of KCN. [1-^{14}C]Palmitoyl-CoA oxidation in the presence of KCN (C). The values represent means ± SD of control animals (0, 7, and 14 days) and means of treated animals.

DHAPAT in the liver for the overall glycerolipid synthesis appears to be minor [16–18]. DGAT and ACAT represent possible sites of regulating plasma triacylglycerol and cholesterol levels. DGAT is located at the branchpoint after which both triacylglycerol and phospholipid formation is possible, whereas ACAT is involved in the secretion of VLDL-cholesterol ester [6]. The mitochondrial and microsomal GPAT activities (Table 3) and DGAT activity (Table 4) were increased in BCMTD- and CMTTD-treated rats, whereas CETTD treatment decreased the above enzyme activities. The ACAT activity was marginally changed in the three experimental groups (Table 4). Thus, changes of DHAPAT, GPAT, DGAT and ACAT activities appear not to be coordinated to neither the development of fatty liver (induced by CETTD) nor the triacylglycerol-lowering effect in both plasma and liver (induced by BCMTD and CMTTD) [3].

Changes of fatty acid oxidation should also be taken into account when considering the mechanism for altered hepatic triacylglycerol content, and thereby changes of plasma lipids. We have recently shown that the hypotriglyceridemia and hypocholesterolemia obtained in BCMTD- and CMTTD-exposed rats were dissociated from induction of peroxisomal β-oxidation and peroxisome proliferation [3]. An increased effect on mitochondrial fatty acid oxidation may be a plausible explanation for a decrease in triacylglycerol and cholesterol efflux from the liver. As can be seen Table 1, CMTTD treatment increased the cyanide-sensitive palmitoyl-CoA oxidation, whereas fatty acid oxidation was dramatically decreased in CETTD-treated rats. This, along with the finding that CMTTD lowered plasma free fatty acids (Berge, data to be published), indicates that the drugs may produce their triacylglycerol-lowering effect by acceleration of mitochondrial fatty acid oxidation and subsequently limiting the availability of free fatty acids for VLDL triacylglycerol synthesis. Furthermore, it is evident that fatty liver formation and increase in plasma triacylglycerol level induced by CETTD might be due to inhibition of mitochondrial fatty acid oxidation (Fig. 2) which in turn increase the amount of fatty acids available for triacylglycerol biosynthesis.

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