Inhibition of hepatic fatty acid oxidation at carnitine palmitoyltransferase I by the peroxisome proliferator 2-hydroxy-3-propyl-4-[6-(tetrazol-5-yl)hexyloxy]acetophenone

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Recent studies suggest that the induction of peroxisomal β -oxidation in rodents may represent an adaptive response to disturbances in hepatic lipid metabolism. The following studies were done to determine the effects of 2-hydroxy-3-propyl-4-[6-(tetrazol-5-yl)hexyloxy]acetophenone (4-THA), a tetrazole-substituted acetophenone which induces peroxisomal β -oxidation in rodent liver, on fatty acid oxidation *in vitro*. In isolated hepatocytes, 4-THA inhibited the oxidation of oleate ($C_{18:1}$) and decreased the mitochondrial redox state. The inhibition was more pronounced in the presence of 0.2 mM-oleate than with 0.5 mM, indicating the inhibition may be competitive. 4-THA had no effect on the oxidation of octanoate ($C_{8:0}$), suggesting that the site of inhibition of oleate oxidation was the carnitine-dependent transport across the mitochondrial inner membrane. In rat liver mitochondria, 4-THA inhibited carnitine palmitoyltransferase I (CPT-I) competitively with respect to the substrate palmitoyl-CoA, increasing the apparent K_m from 19 μ M to 86 μ M. The inhibition of CPT-I by 4-THA was independent of the concentration of the co-substrate carnitine. Whereas fasting attenuated the inhibition of CPT-I by malonyl-CoA, it did not diminish the inhibition by 4-THA. Inhibition of transferase activity by 4-THA and malonyl-CoA was attenuated in mitochondria which had been solubilized with octyl glucoside to expose the latent form of carnitine palmitoyltransferase (CPT-II), suggesting that the inhibition was specific for CPT-I. The specificity was further demonstrated in studies of mitochondrial β -oxidation in which 4-THA inhibited the oxidation of palmitoyl-CoA but not palmitoylcarnitine. The results demonstrate that 4-THA inhibits fatty acid oxidation in rat liver *in vitro* at the site of transport across the mitochondrial inner membrane, CPT-I. Whether this disruption in mitochondrial oxidation is causally related to the induction of peroxisomal β -oxidation is yet to be determined.

INTRODUCTION

A number of dietary and chemical treatments have been found to induce the activity of peroxisomal β -oxidation in rat liver. These include starvation (Ishii *et al.*, 1980), diabetes (Horie *et al.*, 1981), high-fat diets (Neat *et al.*, 1980; Thomassen *et al.*, 1982), hypolipidaemic agents (Lalwani *et al.*, 1983b; Lazarow & de Duve, 1976; Lazarow *et al.*, 1982), phthalate ester plasticizers (Lake *et al.*, 1984; Osumi & Hashimoto, 1978) and leukotriene antagonists with tetrazole-substituted acetophenone structures (Eacho *et al.*, 1986; Eacho & Foxworthy, 1986). These treatments induce numerous peroxisomal and extraperoxisomal enzymes, most of them involved in lipid metabolism. Thus the induction of peroxisomal enzymes may represent an adaptive response to changes in hepatic lipid metabolism (Bremer & Norum, 1982). Increases in hepatic long-chain fatty acyl-CoA elicited by starvation, diabetes (Tubbs & Garland, 1964), high-fat diets (Nilsson *et al.*, 1984; Tubbs & Garland, 1964), hypolipidaemic agents (Bakke & Berge, 1982; Berge & Aarsland, 1985; Skrede & Halvorsen, 1979) and phthalate esters (Shindo *et al.*, 1978) were found to correlate well with induction of peroxisomal

β -oxidation, suggesting that the enzyme induction may be regulated by substrate supply. On the other hand, the hypolipidaemic agents themselves are converted into unmetabolizable CoA esters, which may trigger the induction of peroxisomal β -oxidation (Berge *et al.*, 1987; Bronfman *et al.*, 1986). The very-long-chain fatty acids contained in high-fat diets are converted into CoA esters that are poorly metabolized by mitochondria and inhibit the oxidation of palmitate and oleate (Bremer & Norum, 1982; Christiansen *et al.*, 1977; Osmundsen & Bjornstad, 1985). Metabolites of di-(2-ethylhexyl) phthalate were also found to inhibit mitochondrial β -oxidation (Badr *et al.*, 1985; Elcombe & Mitchell, 1986). These data suggest that the induction of peroxisomal β -oxidation may occur in response to increased substrate supply or inhibited mitochondrial metabolism.

The studies described here were done to investigate the possibility that a disturbance of hepatic lipid metabolism was involved in the induction of peroxisomal β -oxidation caused by the tetrazole-substituted acetophenones (Eacho *et al.*, 1986; Eacho & Foxworthy, 1986). We examined the effect of 2-hydroxy-3-propyl-4-[6-(tetrazol-5-yl)hexyloxy]acetophenone (4-THA) on fatty acid oxidation in isolated rat hepatocytes and mitochondria.

Abbreviations used: 4-THA, 2-hydroxy-3-propyl-4-[6-(tetrazol-5-yl)hexyloxy]acetophenone; CPT, carnitine palmitoyltransferase; POCA, ethyl 2-[5-(4-chlorophenyl)pentyl]oxiran-2-carboxylate.

Fig. 1. Structure of 2-hydroxy-3-propyl-4-[6-(tetrazol-5-yl)hexyloxy]acetophenone (4-THA)

EXPERIMENTAL

Materials

[1-¹⁴C]Oleic acid (57 mCi/mmol), [1-¹⁴C]palmitoyl-CoA (59 mCi/mmol) and L-[1-¹⁴C]palmitoylcarnitine chloride (55 mCi/mmol) were obtained from Dupont-New England Nuclear, Boston, MA, U.S.A. L-[methyl-³H]Carnitine hydrochloride (72 Ci/mmol) was purchased from Amersham, Arlington Heights, IL, U.S.A. Oleic acid, octanoic acid, palmitoylcarnitine, palmitoyl-CoA, L-carnitine, collagenase (type I) and bovine serum albumin (fatty acid-free) were obtained from Sigma Chemical Co., St. Louis, MO, U.S.A. 3-Hydroxybutyrate dehydrogenase was purchased from Boehringer-Mannheim, Indianapolis, IN, U.S.A. 2-Hydroxy-3-propyl-4-[6-(tetrazol-5-yl)hexyloxy]acetophenone (4-THA; Lilly compound 171884; Fig. 1) was synthesized by Dr. W. S. Marshall, Eli Lilly and Co., Indianapolis, IN, U.S.A.

Male Fischer 344 rats weighing approx. 200–250 g were supplied by Harlan Sprague-Dawley, Cumberland, IN, U.S.A. Rats received Purina Rat Chow 5002 and tap water *ad libitum*. When fasted rats were used, food was removed 48 h before they were killed.

Preparation of hepatocytes and mitochondria

Rats were anaesthetized with sodium pentobarbital, and livers were perfused with Ca²⁺-free Krebs-Henseleit buffer (120 mM-NaCl, 5 mM-KCl, 1.2 mM-KH₂PO₄, 1.2 mM-MgSO₄, and 25 mM-NaHCO₃) containing 5 mM-glucose and 0.5 mM-EGTA. Subsequently, livers were perfused with Krebs-Henseleit buffer containing 5 mM-glucose, 4.5 mM-CaCl₂ and 100 units of collagenase/ml, and cells were isolated as previously described (Eacho & Weiner, 1980). Cell viability was greater than 90% for all experiments, as assessed by exclusion of 0.04% Trypan Blue.

Mitochondria were prepared as described previously (Eacho *et al.*, 1985). Solubilization of mitochondria with octyl glucoside was performed as previously described (Woeltje *et al.*, 1987).

Fatty acid oxidation in hepatocytes

Cells (1 × 10⁶ ml) were incubated at 37 °C in Krebs-Henseleit buffer containing 5 mM-glucose, 1.25 mM-CaCl₂, 1.5% bovine serum albumin, 25 mM-Hepes, 0.2 mM- or 0.5 mM-oleate or -octanoate, and 4 mM-L-carnitine. 4-THA was dissolved in 0.5 M-NaHCO₃ and added to cells in a volume of 4 µl/ml. Experiments with [1-¹⁴C]oleate were done in sealed flasks with plastic centre wells containing filter paper spotted with 100 µl of 2 M-KOH. The reaction (2 ml) was terminated by injecting 2 ml of 1 M-HClO₄ through the rubber seal. ¹⁴CO₂ was trapped in the centre well during a 30 min incubation at 37 °C, and the radioactivity of the centre well was counted in 10 ml of Beckman MP scintillation

cocktail. The contents of the flask were centrifuged at 2000 *g* for 15 min and the radioactivity in 1 ml of the acid supernatant was counted. When unlabelled fatty acids were used as substrate, hepatocytes were incubated in open flasks in an atmosphere of O₂/CO₂ (19:1). The reactions were terminated by adding 2 ml of cell incubation mixture to 2 ml of 1 M-HClO₄. After centrifugation, the supernatant was neutralized with 2 M-KOH containing 0.3 M-Mops, and assayed for acetoacetate (Mellanby & Williamson, 1974) and β-hydroxybutyrate (Williamson & Mellanby, 1974).

Mitochondrial fatty acid oxidation

Mitochondrial β-oxidation was determined under the conditions described by Turnbull *et al.* (1984), except that 1-¹⁴C-labelled substrates were used. Approx. 0.3 mg of mitochondrial protein was incubated in 120 mM-KCl/2.5 mM-K₂HPO₄/10 mM-Hepes/5 mM-MgCl₂/1 mM-EDTA, pH 7.2, containing 0.4 mM-ADP, 10 mM-malonnate, 4 mg of bovine serum albumin/ml and 20 µM-[1-¹⁴C]palmitoyl-CoA or -[1-¹⁴C]palmitoylcarnitine (0.5 µCi/µmol). L-Carnitine (0.7 mM) was included when palmitoyl-CoA was used as substrate. 4-THA was added in 5 µl of 0.5 M-NaHCO₃. The reactions (1 ml) were preincubated at 30 °C for 5 min before the addition of radiolabelled substrate. The reaction vessels were then sealed as described for hepatocytes and incubated for 5 min. Reactions were terminated with 1 ml of 1 M-HClO₄, and ¹⁴CO₂ and acid-soluble metabolites were measured as described above.

Assay of CPT-I

CPT-I activity was assayed as described by Gamble & Cook (1985) in a 0.5 ml volume containing 50 mM-imidazole, 60 mM-KCl, 100 mM-sucrose, 35 mM-Hepes, 1 mM-EGTA, 2.5 mM-MgCl₂, 2.5 mM-ATP, 5 mM-GSH, 2.25 mg of bovine serum albumin, 0.5 µg of antimycin A and 0.1 mg of mitochondrial protein. Palmitoyl-CoA was added at 5, 10, 25, 50 or 100 µM and L-[methyl-³H]-carnitine at 20, 50, 100, 200 or 500 µM. The specific radioactivity of [³H]carnitine was kept constant at 5 µCi/µmol. 4-THA was dissolved in 0.5 M-NaHCO₃ and 5 µl was added to the reaction tube. [³H]Palmitoylcarnitine was extracted in butanol as described by Bremer *et al.* (1985).

RESULTS

Isolated hepatocytes

Ketogenesis was linear for 30 min in isolated rat hepatocytes incubated with 0.5 mM-oleic acid (Fig. 2). The addition of 0.5 mM-4-THA decreased ketone production at all incubation times, associated with a decrease in the β-hydroxybutyrate/acetoacetate ratio. At 30 min, the ratio in control cells was 1.22 ± 0.19 (mean ± S.E.M.) compared with 0.58 ± 0.06 in 4-THA-treated cells. Production of acid-soluble metabolites of 0.5 mM-[¹⁴C]oleic acid, representing mainly ketones (Christiansen, 1977), was likewise inhibited by 0.5 mM-4-THA (Table 1). ¹⁴CO₂ production in the same cells was increased by 43%, resulting in a net decrease of 30% in total metabolism (acid-soluble + ¹⁴CO₂). At an oleic acid concentration of 0.2 mM, 4-THA caused a proportionately greater inhibition of the production of acid-soluble metabolites, as well as a decrease in ¹⁴CO₂ production. Total metabolism

Table 1. Inhibition of 0.2 mM- and 0.5 mM-[1-¹⁴C]oleate oxidation by 4-THA in isolated rat hepatocytes

Hepatocytes were incubated in sealed flasks in the presence of 0.2 mM- or 0.5 mM-[1-¹⁴C]oleate (sp. radioactivity 0.16 $\mu\text{Ci}/\mu\text{mol}$) for 30 min. ¹⁴CO₂ and acid-soluble metabolites were measured, and their sum is expressed as 'Total'. Values, expressed as d.p.m./30 min per 10⁶ cells, represent means $\pm$ S.E.M. for four separate experiments. Values in parentheses represent percentages of control.

[1- ¹⁴ C]Oleate concn.	Treatment	[1- ¹⁴ C]Oleate metabolites (d.p.m./30 min per 10 ⁶ cells)		
		¹⁴ CO ₂	Acid-soluble	Total
0.2 mM	Control	1184 $\pm$ 172	3454 $\pm$ 438	4637 $\pm$ 563
	0.5 mM-4-THA	814 $\pm$ 140 (69)	1187 $\pm$ 160 (34)	2001 $\pm$ 274 (43)
0.5 mM	Control	2088 $\pm$ 232	12127 $\pm$ 983	14215 $\pm$ 1057
	0.5 mM-4-THA	2984 $\pm$ 190 (143)	7077 $\pm$ 644 (58)	10061 $\pm$ 637 (71)

of 0.2 mM-[¹⁴C]oleic acid was decreased by nearly 60 % by 4-THA.

Ketogenesis from octanoate was not markedly changed by 4-THA (Table 2). In the presence of 0.5 mM-4-THA, rates of ketogenesis from 0.2 mM- and 0.5 mM-octanoate were 87 and 103 % of control respectively. The lack of effect on ketogenesis from octanoate demonstrates that the functional viability of the cells was not impaired by 4-THA. Assessment of cell viability by the exclusion of Trypan Blue confirmed that treatment with 4-THA did not adversely affect cell viability under any of the conditions studied.

Mitochondria

Because 4-THA specifically inhibited ketogenesis from the long-chain fatty acid, experiments were done to determine if CPT-I was the site of inhibition. At 100 μM -palmitoyl-CoA, the addition of 100 μM -4-THA had little effect on CPT-I activity. At 50 μM -palmitoyl-CoA and

below, 4-THA caused inhibition that was inversely related to the concentration of palmitoyl-CoA (Fig. 3). The K_m for palmitoyl-CoA determined by the Lineweaver-Burk equation was increased from 18.9 μM in control mitochondria to 86.2 μM in the presence of 100 μM -4-THA, indicating that 4-THA is a competitive inhibitor of CPT-I with respect to palmitoyl-CoA. The effect of the co-substrate carnitine was examined because it was found to attenuate the inhibition of CPT-I by malonyl-CoA (Bird & Saggerson, 1985) and 4-hydroxyphenylglyoxylate (Stephens *et al.*, 1985). The inhibition caused by 4-THA in the presence of 10 μM -, 25 μM - or 50 μM -palmitoyl-CoA was independent of carnitine at concentrations ranging from 20 to 500 μM (Table 3).

The effects of 48 h fasting on inhibition of CPT-I by 4-THA and malonyl-CoA are shown in Table 4. Fasting greatly attenuated the inhibition caused by malonyl-CoA, but the inhibition caused by 4-THA was not diminished. The inhibitory effect of 4-THA was further compared with that of malonyl-CoA in mitochondria that were solubilized with octyl glucoside to expose the latent carnitine palmitoyltransferase (CPT-II). As reported by others (Declercq *et al.*, 1985), the malonyl-CoA effect on transferase activity was attenuated under this condition (Table 4). The inhibition by 4-THA was also attenuated in solubilized mitochondria, suggesting that the inhibition was specific for CPT-I.

The specificity of 4-THA was further demonstrated in

Fig. 2. Inhibition of ketogenesis from 0.5 mM-oleate by 0.5 mM-4-THA in isolated rat hepatocytes

The cells were incubated in an atmosphere of O₂/CO₂ (19:1) for 10, 20 or 30 min in the absence (○) or presence (●) of 4-THA. Points and bars represent means $\pm$ S.E.M. for three experiments.

Table 2. Effect of 4-THA on ketogenesis from 0.2 mM- and 0.5 mM-octanoate in isolated rat hepatocytes

Hepatocytes were incubated in an atmosphere of O₂/CO₂ (19:1) for 30 min. Values, expressed as nmol of ketones/30 min per 10⁶ cells, represent means $\pm$ S.E.M. for six separate experiments. Values in parentheses represent percentages of control.

Treatment	[Octanoate]...	Ketogenesis (nmol of ketones/30 min per 10 ⁶ cells)	
		0.2 mM	0.5 mM
Control		227.2 $\pm$ 21.1	521.5 $\pm$ 17.1
0.5 mM-4-THA		197.0 $\pm$ 14.9 (87)	537.1 $\pm$ 21.1 (103)

Fig. 3. Inhibition of CPT-I by 4-THA as a function of palmitoyl-CoA concentration

Mitochondria were preincubated with 100 μM -4-THA in the presence of 5–100 μM -palmitoyl-CoA for 5 min before initiation of the reaction. Values, expressed as percentages of control, represent means for six rats. Control activity in the presence of 5, 10, 25, 50 or 100 μM -palmitoyl CoA was 1.4 ± 0.2 , 2.0 ± 0.3 , 3.6 ± 0.4 , 4.7 ± 0.5 , or 6.7 ± 0.7 nmol/min per mg of protein respectively. Inset: Lineweaver-Burk plot in which v is nmol/min per mg of protein and s is μM -palmitoyl-CoA, in the absence (○) and the presence (●) of 4-THA.

Table 3. Effect of carnitine concentration on the inhibition of CPT-I by 4-THA

The reaction mixture contained 10 μM -, 25 μM - or 50 μM -palmitoyl-CoA. Mitochondria were preincubated in the presence or the absence of 100 μM -4-THA for 5 min before addition of carnitine. Values, expressed as percentages of control activity, represent means for three rats.

[Carnitine] (μM)	[Palmitoyl-CoA] (μM)...	CPT-I (% of control)		
		10	25	50
20		47	69	83
50		49	65	83
100		48	58	78
200		56	64	87
500		45	57	81

studies of mitochondrial β -oxidation using palmitoyl-CoA and palmitoylcarnitine as substrates. β -Oxidation of palmitoyl-CoA was markedly inhibited by 4-THA, whereas β -oxidation of palmitoylcarnitine, which by-passes CPT-I, was only minimally affected (Table 5).

DISCUSSION

The initial objective of this investigation was to examine possible mechanisms by which 4-THA induced peroxisomal β -oxidation in rat liver. The mechanism by

Table 4. Effect of fasting and octyl glucoside solubilization on the inhibition of CPT-I caused by 4-THA and malonyl-CoA

Mitochondria were prepared from fed or 48 h-fasted rats. Solubilization of mitochondria from fed rats was performed with octyl glucoside. The reactions contained 200 μM -carnitine and 10–50 μM -palmitoyl-CoA in the presence or absence of 100 μM -4-THA or 5 μM -malonyl-CoA. Values, expressed as percentages of control, represent means of three to six rats.

Inhibitor	Mitochondria condition	[Palmitoyl-CoA] (μM)...	CPT-I (% of control)		
			10	25	50
4-THA	Fed rats		52	66	86
	Fasted rats		53	65	88
	Solubilized		84	93	105
Malonyl-CoA	Fed rats		3	8	21
	Fasted rats		30	40	56
	Solubilized		90	90	94

Table 5. Effect of 4-THA on β -oxidation of [1- ^{14}C]palmitoyl-CoA and [1- ^{14}C]palmitoylcarnitine in rat liver mitochondria

Mitochondria (0.3 mg of protein) were preincubated for 5 min in the presence or absence of 4-THA. Radiolabelled substrates were then added and reactions were carried out for 5 min. Acid-soluble metabolites and $^{14}\text{CO}_2$ were measured, and activity is presented as nmol of substrate oxidized/min per mg of mitochondrial protein. Values represent means $\pm$ S.E.M. for three rats. Values in parentheses represent percentages of control.

Treatment	β -Oxidation	
	Palmitoyl-CoA	Palmitoyl-carnitine
Control	3.66 ± 0.28	6.94 ± 0.32
100 μM -4-THA	1.41 ± 0.10 (39)	6.06 ± 0.18 (87)

which peroxisomal β -oxidation, and peroxisome proliferation in general, are induced by chemical or other means is not understood. One proposed mechanism involves a cytoplasmic receptor which binds hypolipidaemic agents and subsequently activates the genes that regulate peroxisomal enzyme synthesis (Lalwani *et al.*, 1983a). This concept does not account for the induction of peroxisomal β -oxidation, albeit more modest, caused by fat-feeding (Neat *et al.*, 1980; Thomassen *et al.*, 1982), starvation (Ishii *et al.*, 1980) and diabetes (Horie *et al.*, 1981).

An alternative explanation is that the peroxisomal response is part of an adaptive change in the liver initiated to correct a disturbance in lipid metabolism. Transient lipid accumulation is one such alteration observed shortly after administration of peroxisome-proliferating agents to rats (Mann *et al.*, 1985; Mitchell *et al.*, 1985; Price *et al.*, 1986). Lipid droplets appear within the first 24 h of treatment, and thereafter

disappear from the centrilobular zone where peroxisomes are concentrated. Inhibition of mitochondrial fatty acid oxidation has been proposed as a factor involved in the lipid accumulation and induction of peroxisomal β -oxidation (Badr *et al.*, 1985; Elcombe & Mitchell, 1986). Supporting this proposal is the observation that valproic acid and ethyl 2-[5-(4-chlorophenyl)pentyl]oxiran-2-carboxylate (POCA), both inhibitors of mitochondrial fatty acid oxidation (Olson *et al.*, 1986; Turnbull *et al.*, 1984), induce peroxisomal β -oxidation (Bone *et al.*, 1982; Draye & Vamecq, 1987; Horie & Suga, 1985) and cause lipid accumulation in rodent liver (Koundakjian *et al.*, 1984; Olson *et al.*, 1986). We observed that the peroxisome-proliferating agent 4-THA caused transient lipid accumulation in rat liver (P. S. Foxworthy & P. I. Eacho, unpublished work), so the possibility that 4-THA was an inhibitor of mitochondrial fatty acid oxidation was investigated, with the longer-range objective of determining whether such inhibition was involved in the peroxisomal response.

In isolated rat hepatocytes 4-THA inhibited ketogenesis from the long-chain fatty acid oleate. This was associated with a decrease in mitochondrial [NADH]/[NAD⁺] ratio. The inhibition of ketogenesis as estimated from acid-soluble metabolites of [¹⁴C]oleate was greater with 0.2 mM- than with 0.5 mM-oleate. Likewise, total metabolism (acid-soluble + ¹⁴CO₂) of 0.2 mM-oleate was inhibited more than that of 0.5 mM-oleate, suggestive of competitive inhibition. ¹⁴CO₂ production from 0.5 mM-oleate was increased by 4-THA, whereas it was decreased with 0.2 mM-oleate. The increased ¹⁴CO₂ production may have been attributable to the decrease in mitochondrial redox, which would increase Krebs-cycle activity, or to uncoupling of oxidative phosphorylation, which occurs with weakly acidic peroxisome proliferators (Woods *et al.*, 1977). Since ¹⁴CO₂ formation was not increased at 0.2 mM-oleate, either uncoupling alone did not account for the increase, or it involved the additional uncoupling effects of oleate (Rottenberg & Hashimoto, 1986).

Ketogenesis from octanoate was not inhibited by 4-THA in rat hepatocytes. The data suggested that the site of inhibition of oleate oxidation was the carnitine-dependent transport across the mitochondrial inner membrane, a step that is by-passed in octanoate oxidation. This was confirmed in studies of CPT-I in rat liver mitochondria. 4-THA inhibited CPT-I at palmitoyl-CoA concentrations in the range 5–50 μ M. Kinetic data generated with double-reciprocal plots (1/*v* versus 1/[palmitoyl-CoA]) revealed that 4-THA increased the apparent *K_m* for palmitoyl-CoA without changing the *V_{max}*, indicating that the inhibition was competitive with respect to acyl-CoA substrate. The inhibitory effect of 4-THA was attenuated in octyl glucoside-solubilized mitochondria, a preparation in which mainly CPT-II activity is expressed (Woeltje *et al.*, 1987). This property was shared with malonyl-CoA, the physiological regulator of CPT-I. Therefore, like malonyl-CoA, the effect of 4-THA appears to be directed specifically at CPT-I. The specificity of 4-THA for CPT-I was further illustrated in the comparison of palmitoyl-CoA and palmitoyl-carnitine as substrates for mitochondrial β -oxidation. 4-THA markedly inhibited the oxidation of palmitoyl-CoA, but had little effect on oxidation of palmitoyl-carnitine, which by-passes CPT-I. These data also indicated that 4-THA did not inhibit the matrix enzymes of mitochondrial β -oxidation.

The inhibitory effect of 4-THA was distinguished from that of malonyl-CoA in mitochondria from fasted rats. Fasting significantly attenuated the inhibitory effect of malonyl-CoA on CPT-I (Table 4, and Bremer, 1981), whereas fasting had no effect on the inhibition caused by 4-THA. Furthermore, malonyl-CoA inhibition was found to diminish with increasing concentrations of carnitine (Bird & Saggerson, 1985), whereas the inhibitory effect of 4-THA was independent of carnitine.

The characteristics of 4-THA are similar in some respects to those of other exogenous inhibitors. For example, 2-bromopalmitate inhibits mitochondrial oxidation of palmitoyl-CoA, but not palmitoylcarnitine (Chase & Tubbs, 1972). In addition, the inhibition of CPT-I by the CoA ester of bromopalmitate is attenuated in detergent-solubilized mitochondria (Declercq *et al.*, 1987) but is unaffected by fasting (Edwards *et al.*, 1985). Bromopalmitate, like other CPT-I inhibitors including tetradecylglycidate (Kiorpes *et al.*, 1984) and POCA (Turnbull *et al.*, 1984), contains a carboxylic acid which requires conversion into a CoA ester to produce the active inhibitor (Chase & Tubbs, 1972). 4-THA contains a tetrazole moiety, which, to our knowledge, is not found in previously reported inhibitors of CPT-I. The tetrazole is an acid function that is chemically and physically similar to the carboxylic acid (Thornber, 1979), but it has not been determined if the tetrazole of 4-THA can be converted into a CoA derivative.

Thus 4-THA is an inhibitor of CPT-I in rat liver mitochondria which is structurally unique compared with other known inhibitors. The significance of the inhibition of CPT-I with respect to the induction of peroxisomal β -oxidation in rat liver is uncertain. We have found that, before significant induction of peroxisomal β -oxidation, 4-THA causes transient lipid accumulation in rat liver (P. S. Foxworthy & P. I. Eacho, unpublished work). This effect may be related to inhibition of CPT-I, but whether inhibition of fatty acid oxidation in mitochondria is involved causally in the development of an adaptive response in peroxisomes is a question that requires investigation.

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