Hormonal and substrate regulation of 3-thia fatty acid metabolism in Morris 7800 C1 hepatoma cells

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The 3-thia fatty acid tetradecylthioacetic acid (TTA) has recently been shown to inhibit growth rate and increase peroxisomal acyl-CoA oxidase (ACO) (EC 1.3.99.3) activity in the Morris 7800 C1 hepatoma cells. Dexamethasone potentiates and insulin antagonizes these effects of TTA. We demonstrate here the metabolism of the 3-thia acids in these cells and the influence of insulin and dexamethasone on this. (1) The Morris 7800 C1 hepatoma cells exhibited a low ω -hydroxylation activity of the 3-thia acid (and lauric acid). The combination of TTA and dexamethasone induced the ω -hydroxylation and ACO activities in these cells. TTA alone induced ACO activity, but not ω -hydroxylation activity. Insulin counteracted the induction of both enzyme activities. These results indicate that these two enzyme activities are under similar but independent regulation. (2) Hepatoma cells

grown with 80 μ M TTA in the medium accumulated phospholipids containing the 3-thia fatty acid. After 7 days, TTA accounted for approx. 40% of the total fatty acids in the phospholipids. In addition, TTA affected the incorporation of endogenous fatty acids into phospholipids by decreasing the amounts of palmitic ($C_{16:0}$) and vaccenic ($C_{18:1(n-7)}$) acid and increasing the amounts of linoleic ($C_{18:2(n-6)}$) and α -linolenic ($C_{18:3(n-3)}$) acid in the phospholipids. (3) Dexamethasone increased the incorporation of labelled TTA into both phospholipids and triacylglycerol. Most of the labelled triacylglycerol formed was secreted into the medium. Insulin increased the incorporation of labelled TTA into triacylglycerol, but not into phospholipids. The labelled triacylglycerol formed was retained in the cells.

INTRODUCTION

The sulphur-containing 3-thia fatty acid tetradecylthioacetic acid (TTA) decreases serum triacylglycerol and serum cholesterol when fed to rats [1]. It also induces a series of fatty-acid-metabolizing enzymes in the liver, including the peroxisomal β -oxidation and the microsomal fatty acid ω -hydroxylating enzymes [1,2].

The sulphur atom in the 3-position blocks normal β -oxidation of these acids. They are thus metabolized by ω -oxidation and excreted in the urine as short-chain dicarboxylic sulphoxides [2,3]. In isolated hepatocytes, the 3-thia fatty acids are taken up and incorporated into phospholipids and triacylglycerol at rates which may even exceed those of normal fatty acids, presumably because normal oxidation is blocked [4].

In Morris 7800 C1 hepatoma cells TTA inhibits cell growth and increases the peroxisomal acyl-CoA oxidase (ACO) activity and mRNA level [5–7]. Dexamethasone potentiates and insulin antagonizes these effects of TTA [5,6]. The increase in ACO mRNA level by TTA involves both transcriptional effects and mRNA stabilization [6]. These results suggest that the 7800 C1 Morris hepatoma cells represent an interesting model for studying the metabolism of 3-thia acids and the influence of dexamethasone and insulin on this.

In the present study we have investigated how the induction of ω -hydroxylation activity of 3-thia acid (and lauric acid) is correlated with the induction of ACO activity in the hepatoma cells. We have also investigated to what extent TTA can substitute for normal fatty acids in the cellular phospholipids, and how insulin and dexamethasone influence the incorporation of TTA into triacylglycerol and its excretion from the cells into the medium.

EXPERIMENTAL

Materials

Ham's F-10 medium, horse serum and calf serum were from Flow Laboratories (Irvine, Ayrshire, Scotland, U.K.). Anti-PPLO, fungizone, penicillin and streptomycin were from Gibco (Grand Island, NY, U.S.A.). Dodecylthioacetic acid (DTA) and TTA were synthesized as previously described [4,7]. Other chemicals were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.).

Cell culture

The establishment and propagation of the 7800 C1 hepatoma cell strain has been described previously [8]. The cells were grown in plastic culture dishes (145 mm $\times$ 20 mm) or bottles (75 cm²). The cell medium was Ham's F-10 medium supplemented with 10% horse serum, 3% calf serum, penicillin (50 units/ml), streptomycin (50 μ g/ml), fungizone (2.5 μ g/ml) and anti-PPLO (60 μ g/ml). The cells were incubated at 37 °C in a humidified atmosphere of O₂/CO₂ (19:1) and were grown with the same medium for 5 days after plating. The medium was subsequently changed every second day until the cultures reached plateau phase. The medium was then supplemented with either palmitic acid (80 μ M), TTA (80 μ M), dexamethasone (0.25 μ M), and/or insulin (0.4 μ M). The chosen concentrations of TTA, dexamethasone and insulin were based on previous studies on induction of peroxisomal β -oxidation in the Morris 7800 C1 hepatoma cells [5–7,9]. The cells were treated for 7 days, at which time there is a steady response of the cells to these compounds.

Incubations of the Morris 7800 C1 hepatoma cells with [1-¹⁴C]TTA

The growth medium with additions was removed from the hepatoma cells after 7 days. All dishes were then incubated for 17 h with 10 ml of medium containing 80 μ M [1-¹⁴C]TTA (1.8 μ Ci/ μ mol) and in addition either dexamethasone (0.25 μ M) and/or insulin (0.4 μ M). The incubation was stopped by removing the medium and washing the cells with 2 $\times$ 1.0 ml of 0.15 M KCl/20 mM Hepes buffer (pH 7.4). The wash phases were combined with the medium. The dishes were then scraped with 3.0 ml of the same buffer and routinely sonicated for 15 s with a Sonicator cell disruptor.

To samples of the medium and cell suspensions (450 μ l), 4 M HClO₄ (100 μ l) was added. They were centrifuged to remove lipids and protein and a sample of the acid-soluble phase counted for radioactivity. Other samples of the medium and cell suspensions (2.0 ml) were extracted with butan-1-ol (1.0 ml), as described previously [4], and a sample was counted for radioactivity. The butan-1-ol phase was then removed under a stream of nitrogen, and the residue was resolved in chloroform and stored at -20 °C for subsequent t.l.c. The recovery of radioactivity was close to 100 %.

The butanol-extracted lipids were separated by chromatography on silica-gel aluminium t.l.c. plates with hexane/diethyl ether/acetic acid (35:15:1, by vol.), and the subclasses of phospholipids were separated on t.l.c. using a mobile phase of chloroform/methanol/acetic acid/water (65:25:4:4, by vol.) as described previously [4].

Fatty acid composition of cell lipids

The butanol extracts of two dishes were combined, and to each pooled sample was added 50 μ g of L- α -diheptadecanoyl phosphatidylcholine as internal standard. The butanol was removed under a stream of nitrogen and the residues were dissolved in chloroform. Each sample was then subjected to anion-exchange chromatography on bonded-phase columns, as described by Kaluzny et al. [10], to yield non-esterified fatty acid, triacylglycerol and phospholipid fractions. The phospholipid fraction was hydrolysed in methanolic 15 % (w/v) KOH for 45 min at 65 °C, and the non-esterified fatty acids were liberated by acidification with 6 M HCl, and then extracted with hexane. The fatty acids were then converted into picolinyl esters as described by Christie [11] and separated by capillary gas chromatography on a 50 m BP1 column (0.22 mm internal diam.; S.G.E. International, Ringwood, Victoria, Australia) using a Carlo Erba Model 4150 gas chromatograph fitted with a flame ionization detector and a Shimadzu C-R6A Chromatopac electronic integrator. After splitless sample injection, the column was initially held for 3 min at 40 °C, then programmed to increase at 40 °C/min to 140 °C, held there for 3 min, then programmed to increase at 40 °C/min to 280 °C. Amounts of TTA and other fatty acids were calculated by using the internal standard method. The amount of TTA integrated proved to be only half of the actual amount of the 3-thia acid. This was corrected for.

The identity of the TTA peak was verified by subjecting parallel samples to gas chromatography-mass spectrometry on a Shimadzu GCMS QP-2000 instrument equipped with a 40 m DB1 column (0.18 mm internal diam.; J&W Scientific, Folsom, CA, U.S.A.).

ω -Hydroxylation and sulphur oxygenation of [1-¹⁴C]DTA

The sonicated cell suspensions were incubated as described previously [12]. The incubation mixture contained 65 mM Tris

buffer, pH 7.4, 0.7 mM NADPH, 45 μ M [1-¹⁴C]DTA (5 μ Ci/ μ mol) and 30–90 μ g of DNA of cells. Total volume was 2.0 ml, the temperature 37 °C and the incubation time 15.5 min. The incubation was started with DTA and stopped with 0.8 ml of acetonitrile, and cellular protein was removed by centrifugation. The radioactive products were analysed by h.p.l.c. using a reverse-phase column, Supelcosil LC-18 (Supelco, Bellefonte, PA, U.S.A.) connected to a Raytest Ramona 5LS liquid-scintillation counter (Isotopenmessgeräte, Straubenhardt, Germany). The products were separated as described previously [2].

ω -Hydroxylation of [1-¹⁴C]lauric acid

The cells were treated and incubated as above, except that the incubation mixture contained 25 mM Hepes, pH 7.4, and 60 μ M [1-¹⁴C]lauric acid (3.6 μ Ci/ μ mol). The incubation was stopped with 0.8 ml acetonitrile. To separate ω - and (ω - 1)-hydroxylated products, the acetonitrile was evaporated under nitrogen and 0.8 ml of methanol was added before analysis of the products on h.p.l.c., using the same column and liquid scintillator as above. The radioactive products of lauric acid were separated with an isocratic gradient of methanol/acetic acid/water (100:1:100, by vol.) at a flow rate of 0.8 ml/min essentially as described previously [13].

Other assays

DNA was measured as described previously [14].

ACO (palmitoyl-CoA oxidase) was measured as described by Small et al. [15] and modified as previously described [7]. The enzymatic activity was determined in 0.1 M Tris buffer, pH 8.5, including BSA (0.6 %) and FAD (16 μ M), and omitting *NN'*-dimethylformamide and aminotriazole.

Total phospholipids were measured as lipid P [16].

Statistics

Student's *t* test was used for statistical analysis.

RESULTS

Induction of the ω -hydroxylation pathway in the hepatoma cells

When measuring ω -hydroxylation and sulphur oxygenation activity of the 3-thia fatty acids in the Morris 7800 C1 hepatoma cells, DTA was used as substrate, because we have already established a h.p.l.c. system for separation of the initial metabolites of DTA [2]. TTA gives similar results.

Figure 1 Structures of the various thia-substituted fatty acids and their metabolites

(a) DTA; (b) dodecylsulphoxyacetic acid (sulphur-oxygenated DTA); (c) ω -hydroxy-dodecylthioacetic acid (ω -hydroxylated DTA); (d) TTA.

Table 1 Induction of ω -hydroxylation and sulphur oxygenation of DTA in Morris 7800 C1 hepatoma cells

Morris 7800 C1 hepatoma cells were treated with 80 μ M TTA, 0.25 μ M dexamethasone and 0.4 μ M insulin in different combinations for 7 days as described in the Experimental section. The cells were harvested, sonicated, and 30–90 μ g of DNA of cells were incubated with 45 μ M [$1\text{-}^{14}\text{C}$]DTA for 15.5 min to estimate ω -hydroxylation and sulphur oxygenation of DTA. ACO (palmitoyl-CoA oxidase) was measured with 60–70 μ M palmitoyl-CoA and 0.1–0.3 μ g of DNA of cells. The values are given as nmol/min per mg of DNA of substrate oxidized and are means $\pm$ S.E.M. of three dishes: * $P < 0.05$; † $P < 0.005$; ‡ $P < 0.0005$.

Treatment	Activity (nmol/min per mg of DNA)		
	ACO	ω -Hydroxylation	Sulphur oxygenation
Control	175 $\pm$ 10	0.63 $\pm$ 0.04	10.5 $\pm$ 1.0
TTA	1120 $\pm$ 60‡	0.88 $\pm$ 0.16	7.9 $\pm$ 0.4
TTA + dexamethasone	2200 $\pm$ 105‡	4.44 $\pm$ 0.43†	5.4 $\pm$ 0.7*
TTA + insulin	745 $\pm$ 15‡	0.58 $\pm$ 0.17	8.0 $\pm$ 0.5
TTA + insulin + dexamethasone	875 $\pm$ 60‡	1.20 $\pm$ 0.13*	5.9 $\pm$ 0.6*
Dexamethasone	200 $\pm$ 30	1.51 $\pm$ 0.17*	10.5 $\pm$ 0.4

Table 2 Fatty acids in standard growth medium of Morris 7800 C1 hepatoma cells

The values represent the total fatty acids (in μ M) in the standard growth medium of Morris 7800 C1 hepatoma cells, and are given as means $\pm$ S.E.M. of three different measurements; n.d., not detectable.

Fatty acid	Concn. (μ M)
C _{14:0}	19.4 $\pm$ 3.4
C _{16:1} (n-7)	82.7 $\pm$ 7.0
C _{16:0}	6.2 $\pm$ 2.6
C _{18:3} (n-3)	21.8 $\pm$ 3.6
C _{18:2} (n-6)	175.4 $\pm$ 22.8
C _{18:1} (n-9)	77.4 $\pm$ 7.8
C _{18:1} (n-7)	6.2 $\pm$ 0.7
C _{18:0}	85.7 $\pm$ 5.1
C _{20:4} (n-6)	1.2 $\pm$ 0.1
C _{20:5} (n-3)	n.d.

Figure 2 Induction of ω -hydroxylation of lauric acid by TTA in 7800 C1 hepatoma cells

Morris hepatoma cells were treated for 1, 3 and 7 days with 80 μ M TTA and 0.25 μ M dexamethasone as described in the text. ACO (palmitoyl-CoA oxidase) (a) was measured with 60–70 μ M palmitoyl-CoA and 0.5–1.0 μ g of DNA of cells. ω -Hydroxylation of lauric acid (b) was measured with 60 μ M lauric acid and 70–160 μ g of DNA of cells for 15 min. The values represent two experiments for each time and are presented as nmol of oxidized substrate/min per mg of DNA, and both values are presented: □, control; △, 80 μ M TTA; ◇, 80 μ M TTA + 0.25 μ M dexamethasone.

In the Morris 7800 C1 hepatoma cells [$1\text{-}^{14}\text{C}$]DTA is converted into both sulphur-oxygenated and ω -hydroxylated products in the presence of NADPH (see Figure 1 for the structures of the thia-substituted fatty acids and their metabolites). The sulphur-oxygenation activity was approx. 15 times greater than the ω -hydroxylation activity (Table 1). In contrast, the two activities are about equal in rat liver microsomes [2]. In isolated rat hepatocytes the activities of ω -hydroxylation and sulphur oxygenation of DTA are equal, and are approx. 6 times more and 2.5 times less, respectively, compared with the hepatoma cells (results not shown).

Pretreating the cells for 7 days with TTA (80 μ M) did not significantly increase the total ω -hydroxylation of DTA (Table 1). In contrast, the ACO activity increased 6.5-fold. Pretreating the cells with TTA and dexamethasone (0.25 μ M) in combination significantly increased (7-fold) the ω -hydroxylation of DTA and, as shown previously [6], dexamethasone acted synergistically with TTA on the ACO activity (increased 13-fold) (Table 1). Insulin (0.4 μ M) antagonized the inductive effect of TTA and dexamethasone on both the ω -hydroxylation activity and the ACO activity. Dexamethasone alone significantly increased (2.4-fold) the ω -hydroxylation activity, whereas the ACO activity was unchanged (Table 1).

The sulphur oxygenation of DTA was moderately decreased after treatment with TTA (Table 1).

The preferred substrate for the cytochrome *P*-450IVA1 enzyme is lauric acid [17]. Figure 2 shows that the total ω -hydroxylation of lauric acid varied in the same way as the total ω -hydroxylation of DTA. It did not increase in cells treated with TTA, as opposed to the ACO activity. Treating the cells with TTA and dexamethasone in combination increased the ω -hydroxylation activity, with a maximum after 3 days.

The response of the ω -hydroxylation enzyme to pretreatment with TTA alone varied with the age of the stem culture (results not shown). In young stem cultures the ω -hydroxylation was more responsive to TTA alone, but in old cultures dexamethasone was required in addition to induce the enzyme, as shown in Table 1.

Incorporation of TTA into phospholipids

In order to study the extent of TTA incorporation into the lipids in the hepatoma cells, the cells were grown with 80 μ M TTA in the medium for 7 days. Table 3 shows that TTA became the

Table 3 Fatty acid composition of phospholipids in Morris 7800 C1 hepatoma cells after 7 days of TTA in the growth medium

The cells were either grown in standard medium (control) or medium supplemented with 80 μ M of either TTA or palmitic acid for 7 days. The values are given as nmol of fatty acids in the phospholipid fraction/ μ g of DNA and are means $\pm$ S.E.M. of three different experiments: *B, C versus A, $P < 0.01$; †B, C versus A, $P < 0.001$; ‡B, C versus A, $P < 0.0001$.

Fatty acid	Composition (nmol/ μ g of DNA)		
	Control (A)	TTA (B)	Palmitic acid (C)
C _{14:0}	0.22 $\pm$ 0.03	0.41 $\pm$ 0.06	0.63 $\pm$ 0.29
C _{16:1} (n-7)	0.42 $\pm$ 0.03	0.43 $\pm$ 0.009	2.21 $\pm$ 0.07‡
C _{16:0}	1.80 $\pm$ 0.10	0.86 $\pm$ 0.14*	0.70 $\pm$ 0.09*
C _{18:3} (n-3)	0.03 $\pm$ 0.003	0.09 $\pm$ 0.01*	0.05 $\pm$ 0.006
C _{18:2} (n-6)	0.42 $\pm$ 0.04	0.94 $\pm$ 0.06*	0.65 $\pm$ 0.04
C _{18:1} (n-7)	0.84 $\pm$ 0.03	0.91 $\pm$ 0.09	1.10 $\pm$ 0.04*
C _{18:1} (n-7)	0.32 $\pm$ 0.02	0.05 $\pm$ 0.002‡	0.29 $\pm$ 0.003
C _{18:0}	0.50 $\pm$ 0.001	0.42 $\pm$ 0.02	0.65 $\pm$ 0.01†
C _{20:4} (n-6)	0.42 $\pm$ 0.08	0.46 $\pm$ 0.07	0.40 $\pm$ 0.07
C _{20:5} (n-3)	0.15 $\pm$ 0.01	0.16 $\pm$ 0.03	0.18 $\pm$ 0.005
TTA		3.63 $\pm$ 0.51	
TTA-X§		1.04 $\pm$ 0.18	

§ Unknown metabolite of TTA.

Table 4 Effects of TTA and palmitate on phospholipid content in Morris 7800 C1 hepatoma cells

Morris 7800 C1 hepatoma cells were grown with standard medium or 80 μ M of either TTA or palmitic acid for 7 days, and the total amount of phospholipids was measured as lipid P. The values are given as nmol of phospholipid/ μ g of DNA and are for two dishes.

Treatment	Phospholipid content (nmol/ μ g of DNA)
Control	5.7, 5.4
Palmitate	6.0, 5.4
TTA	8.7, 8.8

major fatty acid in the phospholipid fraction of these cells, accounting for nearly 40 % of the total fatty acids. TTA also affected the incorporation of endogenous fatty acids in the phospholipids. Linoleic acid (C_{18:2} (n-6)) and α -linolenic acid (C_{18:3} (n-3)) were significantly increased (Table 3). The medium was relatively rich in linoleic acid (Table 2). It is therefore evident

that TTA increased the uptake of this fatty acid. Palmitic acid (C_{16:0}) and vaccenic acid (C_{18:1} (n-7)) were significantly decreased (Table 3). Treating the cells with 80 μ M palmitic acid also surprisingly decreased the amount of this fatty acid in the phospholipid fraction, but a significant increase in palmitoleic acid (C_{16:1} (n-7)) was found (Table 3). The absolute amount of fatty acids in the phospholipid fraction was nearly doubled in the TTA-treated cells (Table 3), indicating increased phospholipid synthesis. This was confirmed by measurement of the phospholipid content in TTA-treated cells. The amount of phospholipids was increased after 7 days with TTA in the medium (Table 4).

The chromatogram from the cells treated with TTA (results not shown) shows the presence of an unidentified peak. This is presumably a metabolite of TTA, since it was not found in the phospholipid fraction of cells treated with standard medium. The retention time suggests monounsaturated TTA.

Metabolism of [1-¹⁴C]TTA

We also studied the rate of incorporation of [1-¹⁴C]TTA (80 μ M) in shorter experiments (17 h). T.l.c. showed that [1-¹⁴C]TTA was incorporated into phospholipids, di- and tri-acylglycerol (Table 5). Most of the activity was found in the phospholipid fraction (90 %), and very little was found incorporated into di- and tri-acylglycerol (1–2 %). In phospholipids most of the activity was found as phosphatidylcholine (approx. 60 %) and phosphatidylethanolamine (approx. 22 %). No sulphur-oxygenated metabolites were found in the lipid fraction from the cells (results not shown). In the medium, 9 % of the added radioactivity was recovered as acid-soluble metabolites, presumably dicarboxylic sulphoxides (not identified).

Incorporation of [1-¹⁴C]TTA into triacylglycerol

Table 5 shows that pretreatment of the cells with dexamethasone (0.25 μ M) increased the incorporation of labelled TTA into both phospholipids and into di- and tri-acylglycerol. Most of the labelled triacylglycerol formed in the dexamethasone-treated cells was excreted into the medium. The excretion in these cells was increased 4–5-fold. In contrast, when TTA (80 μ M) was present with dexamethasone before incubation with labelled TTA, the labelled triacylglycerol formed was retained in the cell.

Pretreatment of the cells with insulin (0.4 μ M) selectively increased incorporation of labelled TTA into triacylglycerol, without any significant effect on total uptake of TTA into the cells (Table 5). The labelled triacylglycerol formed in the insulin-

Table 5 Effect of pretreatment with TTA and hormones on the incorporation of [1-¹⁴C]TTA into complex lipids in Morris 7800 C1 hepatoma cells

The hepatoma cells were treated with 80 μ M TTA, 0.25 μ M dexamethasone and/or 0.4 μ M insulin in the medium for 7 days as described in the text and then incubated with 80 μ M [1-¹⁴C]TTA for 17 h. The cell and medium fractions were extracted with butan-1-ol and the lipids separated by t.l.c. as described in the text. The values are given as nmol of TTA incorporated into lipids/mg of DNA, and are means $\pm$ S.E.M. for the numbers of dishes in parentheses: * $P < 0.05$; † $P < 0.005$; ‡ $P < 0.0005$.

Treatment	Cells			Medium
	Phospholipid	Diacylglycerol	Triacylglycerol	Triacylglycerol
Control (6)	667 $\pm$ 105	12 $\pm$ 1	12 $\pm$ 2	(4) 16 $\pm$ 2
TTA (4)	861 $\pm$ 183	10 $\pm$ 1	6 $\pm$ 0.5	(2) 18 $\pm$ 6
TTA + dexamethasone (6)	1646 $\pm$ 227†	25 $\pm$ 2‡	89 $\pm$ 19†	(4) 16 $\pm$ 5
Dexamethasone (4)	1498 $\pm$ 52‡	29 $\pm$ 1‡	26 $\pm$ 2‡	(4) 75 $\pm$ 9‡
TTA + insulin (6)	1016 $\pm$ 115*	12 $\pm$ 1	29 $\pm$ 2‡	(4) 14 $\pm$ 1
Insulin (4)	650 $\pm$ 43	12 $\pm$ 3	137 $\pm$ 24‡	(4) 12 $\pm$ 1

treated cells was retained in the cells and not excreted into the medium. The increased incorporation of labelled TTA into triacylglycerol in the insulin-treated cells was decreased when TTA was present in the medium with insulin.

Pretreatment of the cells with unlabelled TTA (80 μ M) in the medium did not prevent the subsequent uptake of labelled TTA, showing that the uptake was a continuous process (Table 5).

DISCUSSION

Induction of the ω -oxidation pathway

We have previously shown that ω -hydroxylation of DTA is induced by TTA in a manner similar to ω -hydroxylation of lauric acid in rat liver microsomes, indicating that ω -hydroxylation of DTA is catalysed by the inducible cytochrome *P*-450IVA1 enzyme [2]. It has been proposed that induction of the microsomal ω -hydroxylase is a prerequisite for the induction of the peroxisomal β -oxidation enzymes [18–20].

Our results suggest that ω -hydroxylation and peroxisomal β -oxidation are regulated in a similar way, since both are induced by TTA and dexamethasone in combination and antagonized by insulin (Table 1 and Figure 2). However, as TTA alone had no significant effect on a very low ω -hydroxylation activity, in contrast with the ACO activity, peroxisomal induction most likely does not depend on a primary induction of ω -hydroxylation.

Incorporation of TTA into phospholipids

The extensive accumulation of TTA and its metabolite in the cell phospholipids (close to 50 % of total fatty acids) shows that 3-thia fatty acids have biological properties surprisingly similar to those of normal fatty acids. This is also shown by the fact that these cells continue to grow, although at a reduced rate [5]. With insulin in the medium the growth rate is even close to normal [5]. The accumulation of TTA-containing phospholipids in the hepatoma cells (Table 3) is probably a result of a slow breakdown of the 3-thia fatty acids, due to the poor ω -hydroxylation activity in these cells.

TTA seems to substitute mainly for palmitic acid in the phospholipid fraction, and apparently displaces this fatty acid into other complex lipids, as TTA did not decrease the total amount of cellular palmitic acid (results not shown). The stimulating effects of TTA on the incorporation of linoleic ($C_{18:2(n-6)}$) and α -linolenic acid ($C_{18:3(n-3)}$) in the phospholipids (Table 3) is most likely due to an increased uptake of these fatty acids, because the same stimulation was found when measuring total cellular fatty acids (results not shown). Conversely, the decreased incorporation of vaccenic acid ($C_{18:1(n-7)}$) in the phospholipids is apparently due to an inhibition of the uptake of this fatty acid by TTA.

Hormonal regulation of the incorporation of TTA into complex lipids

Hypolipidaemic compounds such as the $n-3$ fatty acids inhibit both synthesis and secretion of cellular triacylglycerol [21–24]. In this study, TTA decreases both the increased synthesis of TTA-labelled cellular triacylglycerol found with insulin and the increased secretion of TTA-labelled triacylglycerol found with dexamethasone (Table 5).

The increased synthesis and secretion of TTA-labelled triacylglycerol found with dexamethasone and the increased synthesis

of TTA-labelled cellular triacylglycerol found with insulin (Table 5) are in agreement with several other studies which show the same effects of dexamethasone [25–27] and insulin [28–34].

In conclusion, due to the massive incorporation of TTA into phospholipids in the Morris 7800 C1 hepatoma cells, the cells are evidently a good model system for studying the effects of this on, e.g., the signal-transduction pathway involving phospholipid breakdown. The present results also show that the hepatoma cells are of interest for studying the influence of insulin and dexamethasone on the triacylglycerol-lowering effects of hypolipidaemic compounds.

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