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Co-ordinate induction of hepatic mitochondrial and peroxisomal carnitine acyltransferase synthesis by diet and drugs

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The present studies examined the effect of agents that induce peroxisomal and mitochondrial β-oxidation on hepatic mitochondrial carnitine palmitoyltransferase (CPT) and peroxisomal carnitine acyltransferase [CPT_s of Ramsay (1988) Biochem. J. **249**, 239–245; COT of Farrell & Bieber (1983) Arch. Biochem. Biophys. **222**, 123–132 and Miyazawa, Ozasa, Osumi & Hashimoto (1983) J. Biochem. **94**, 529–542]. In the first studies, high fat diets containing corn oil or fish oil were used to induce peroxisomal and mitochondrial enzymes. Rats were fed one of three diets for 4 weeks: (1) low fat, with corn oil as 11% of energy (kJ); (2) high fat, with corn oil as 45% of kJ; (3) high fat, with fish oil as 45% of kJ. At the end of 4 weeks, both mitochondrial CPT and peroxisomal CPT_s exhibited increases in activity, immunoreactive protein, mRNA levels and transcription rates in livers of rats fed either high-fat diet compared to the low fat diet. Riboflavin deficiency or starvation for 48 h also increased the peroxisomal CPT_s mRNA. A second set of studies used the plasticizer 2-(diethylhexyl)phthalate (DEHP), 0.5% clofibrate or 1% acetylsalicylic acid (fed for 3 weeks) to alter peroxisomal and mitochondrial fatty acid oxidation. With DEHP, the mitochondrial CPT and peroxisomal CPT_s activity, immunoreactive protein, mRNA levels and transcription rate were all increased by 3–5-fold. The peroxisomal CPT_s activity, immunoreactive protein, mRNA levels and transcription rate were increased 2–3-fold by clofibrate and acetylsalicylic acid, again similar to mitochondrial CPT. The results of the combined studies using both diet and drugs to cause enzyme induction suggest that the synthesis of the carnitine acyltransferases (mitochondrial CPT and peroxisomal CPT_s) may be co-ordinated with each other; however, the co-ordinate regulatory factors have not yet been identified.

INTRODUCTION

The mitochondria normally account for 80–95% of cellular fatty acid oxidation (Mannaerts *et al.*, 1979; Veerkamp & van Moerkerk, 1987), but various diet and drug treatments can increase total cellular fatty acid oxidation and change the relative contributions of peroxisomes and mitochondria (Kahonen, 1976, 1979; Markwell *et al.*, 1977; Mannaerts *et al.*, 1978, 1979). The consumption of a high fat diet, especially one rich in fish oil, causes peroxisomal proliferation and increases the contribution of peroxisomal β-oxidation to total cellular β-oxidation of fatty acids (Neat *et al.*, 1980; Thomassen *et al.*, 1982). The activity and synthesis of some of the enzymes of β-oxidation in both peroxisomes and mitochondria are also correlated with changes in β-oxidation due to diet and drugs (Mannaerts *et al.*, 1979; Krahling & Tolbert, 1982; Suga *et al.*, 1982; Brady *et al.*, 1986*b*). Drugs such as clofibrate and the plasticizer 2-(diethylhexyl)phthalate (DEHP) have been shown to induce both peroxisomal and mitochondrial β-oxidative enzymes (Ishii & Suga, 1979; Ozasa *et al.*, 1983; Reddy *et al.*, 1986; Brady & Brady, 1989*b*).

Mitochondria contain two pools of carnitine palmitoyltransferase (CPT) activity, an outer CPT acting on substrates outside of the mitochondrial matrix, and an inner CPT activity, acting on substrates within the mitochondrial matrix. It has not yet been determined conclusively whether the outer and inner CPT activities result from the same protein. The CPT on the outside of mitochondria has been described as the rate-limiting enzyme in hepatic mitochondrial β-oxidation of fatty acids (McGarry & Foster, 1980), and our previous data show that hepatic CPT activity (outer, inner and total) increases in starvation, diabetes and riboflavin deficiency, and that the increased activity is correlated with increased CPT mRNA translation, increased CPT mRNA levels, and increased transcription of the CPT mRNA from DNA for the 68 kDa mitochondrial CPT (Brady *et al.*, 1985, 1988; Brady & Brady, 1987, 1989*a*). CPT activity (total) also increases with increased dietary fat (Brady *et al.*, 1986*b*; Berge *et al.*, 1987), and CPT activity and synthesis increase with DEHP, acetylsalicylic acid and clofibrate administration (Ozasa *et al.*, 1983; Brady & Brady, 1989*b*).

The CPT activity that reacts with substrates outside of the mitochondrial inner membrane is controlled by short-term regulation by malonyl-CoA, and potentially also by long-term regulation of the enzyme via synthesis if the 68 kDa mitochondrial CPT has activity on the outer side

Abbreviations used: DEHP, 2-(diethylhexyl)phthalate; CPT, mitochondrial carnitine palmitoyltransferase [in this paper, CPT refers to the total mitochondrial CPT without distinguishing its mitochondrial location as 'outer' or 'inner' CPT. This CPT is a mitochondrial protein that, in purified form or in isolated mitochondria, does not cross-react with the peroxisomal CPT_s antibody. The cloning of this CPT has been described (Brady *et al.*, 1988)]. CPT_s, peroxisomal carnitine acyltransferase [this enzyme has been described by Ramsay (1988) as CPT_s. It is the enzyme described by Chatterjee *et al.* (1989) and called carnitine octanoyltransferase and it may be the same enzyme as described by Farrell & Bieber (1983) and Miyazawa *et al.* (1983) and called carnitine octanoyltransferase (COT)].

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of the inner membrane (McGarry & Foster, 1980; Brady *et al.*, 1988; Brady & Brady, 1989*a*,*b*). The carnitine acyltransferase (CPT_s) of isolated peroxisomes is also regulated by malonyl-CoA (Ramsay & Derrick, 1988), but little is known of the regulation of peroxisomal CPT_s by diet or drugs. However, synthesis of peroxisomal CPT_s does increase when DEHP is administered (Ozasa *et al.*, 1983; this enzyme was called carnitine octanoyltransferase [COT] by Ozasa *et al.*, 1983).

In the present studies, we wished to determine whether the synthesis of mitochondrial total CPT and the peroxisomal CPT_s of Ramsay (1988) are co-ordinately induced by diet or by drugs which induce peroxisomal and mitochondrial fatty acid oxidation. Thus, we measured mRNA levels and transcription rates of both enzymes in livers of: (1) rats fed high carbohydrate control diet versus rats fed either corn oil or menhaden oil as 45% of energy; (2) rats fed riboflavin-sufficient or -deficient diets; (3) rats fed control chow versus chow with 2% DEHP, 1% acetylsalicylic acid or 0.5% clofibrate. The results suggest that the synthesis of the enzymes in the two organelles is indeed co-ordinated under most conditions.

MATERIALS AND METHODS

Animals and diets

Male weanling Sprague–Dawley rats were obtained from Bio-Labs, St. Paul, MN, U.S.A. In the first experiment, fifteen rats were initially weighed and allotted to one of three groups (five per group): control high carbohydrate diet with corn oil providing 11% of kJ, corn oil providing 45% of kJ, or menhaden oil providing 45% of kJ. The basal diets were obtained from Teklad Test Diets, Madison, WI, U.S.A.; corn oil was Mazola obtained from a local grocer; menhaden oil was kindly given by Mr. Tony Bimbo, Zapata-Haynie Corp., Reedville, VA, U.S.A. The basal diet composition (g/kg) was: 200 g of casein, 3 g of L-methionine, 552 g of sucrose, 150 g of corn starch, 50 g of corn oil, 35 g of mineral mix and 10 g of vitamin mix. This diet was described in more detail in past work and contains 16.57 kJ/g (3.96 kcal/g) (Brady *et al.*, 1986*b*). To alter this diet to a high fat diet with the same kJ/g, the following modifications were made: 422.3 g of sucrose, 145.2 g of corn starch, 20 g of corn oil, 179.5 g of corn oil or fish oil and 74.7 g of cellulose. Animals were allowed food and water *ad libitum* for four weeks, when the experiment was terminated. Animals were weighed weekly and there was no difference in mean body weight between groups during the course of the experiment. Two replications of this original design were run separately using three rats/group to obtain data on peroxisomal long-chain carnitine acyltransferase activity and immunoreactive protein. In the second experiment, rats were fed either chow, chow with 2% DEHP, chow with 1% acetylsalicylic acid, or chow with 0.5% clofibrate for 3 weeks. In one experiment, an acute glucagon injection (150 μg/kg) was given to control chow-fed and DEHP-fed rats, and animals were killed 3 h later. Mitochondria were isolated as previously described (Hoppel *et al.*, 1979), but without the extensive washes. Peroxisomes were isolated as described (Brady & Hoppel, 1983). There was no cross-reactivity of the anti-(peroxisomal CPT_s) antibody with the mitochondria, nor of the anti-(mitochondrial CPT) antibody with peroxisomes in these studies.

Carnitine palmitoyltransferase activity, mRNA determination and transcription rates

Total CPT activity and translation were determined as described in detail, but at 25 °C without detergent addition (Brady & Brady, 1987). This procedure used detergent-solubilized mitochondria, and substrate concentrations of 40 μM-palmitoyl CoA and 8 mM-L-carnitine. Blanks were run without L-carnitine addition to subtract the acyl-CoA hydrolase activity. Immunoreactive CPT was determined as described in detail (Brady *et al.*, 1988). The mitochondrial CPT that was purified, used to generate antibody and used to generate the CPT clone was the 68 kDa mitochondrial CPT. This CPT is synthesized as a larger precursor (71.5 kDa) by reticulocyte lysate translation (Ozasa *et al.*, 1983; Brady & Brady, 1987). The activity, immunoreactive protein, transcription and mRNA levels of this CPT have been found to increase in starvation, diabetes and riboflavin deficiency, and when clofibrate and aspirin are fed (Brady *et al.*, 1988; Brady & Brady, 1989*a*,*b*).

The procedures for RNA isolation and Northern blots and transcription assays have been described in detail (Marzluff & Huang, 1984; Morris *et al.*, 1987; Brady *et al.*, 1988; Brady & Brady, 1989*a*).

Peroxisomal carnitine acyltransferase activity, immunoreactive protein, mRNA translation and quantification, and transcription rates

Peroxisomal CPT_s was purified, and polyclonal antibodies were generated in rabbits as described (Ramsay *et al.*, 1987; Ramsay, 1988). CPT_s activity was determined as described for CPT activity, except that octanoyl-CoA was substituted for palmitoyl-CoA. The e.l.i.s.a. was performed as for CPT above, with the peroxisomal protein as the antigen. Western blots for quantification of protein were as described (Brady & Brady, 1987). In addition, poly(A)$^+$ RNA was isolated from total RNA as described and then translated (Brady & Brady, 1987). Anti-(peroxisomal CPT_s) was reacted with the translate and then subjected to SDS/polyacrylamide-gel electrophoresis, followed by autoradiography (Brady & Brady, 1987). Fig. 1 shows translated CPT_s precursor, of approx. molecular mass 69.5 kDa. The weight of this nascent translated protein was the same as that of the mature protein under our SDS/polyacrylamide-gel electrophoresis conditions.

The same rat liver library in λgt11, as described for CPT (Brady *et al.*, 1988), was screened for the peroxisomal CPT_s using the anti-(peroxisomal CPT_s) antibody. Concurrently, the amino acid sequence of one tryptic peptide of the purified protein was determined, and a partial nucleotide sequence of the clone $pBluescript_{ROX}$ determined. The amino acid sequence (IPGITR) of the tryptic peptide matched an amino acid sequence deduced from the nucleotide sequence (100% identical) by dideoxy sequencing using *Sequenase* (Maniatis *et al.*, 1982). A 17-mer oligoprobe with a degeneracy of 256 was prepared based on this amino acid sequence and used for re-screening the clone and for Northern and dot-blot analyses. A 45-mer probe was generated based on a second nucleotide sequence obtained from $pBluescript_{ROX}$ (which also matched that of Chatterjee *et al.*, 1988) and was also used for Northern and dot-blots. A lysogen-derived β-galactosidase fusion protein was also synthesized; the deduced molecular

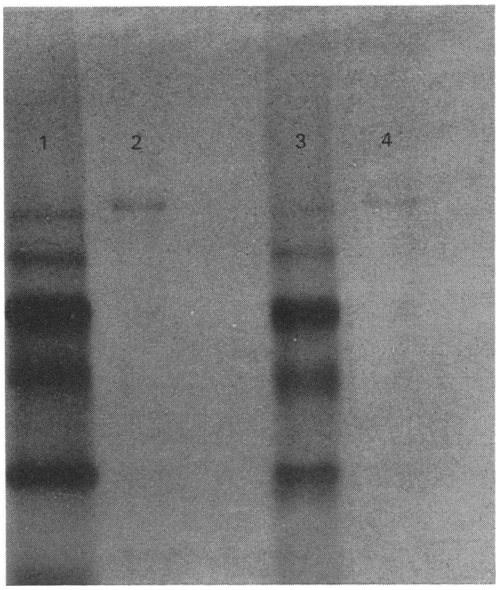

Fig. 1. Translation of hepatic CPT_s showing molecular mass of precursor

Total hepatic RNA was extracted and translated as described in the Materials and methods section and in Brady & Brady (1987). The translated [^{35}S]Met-labelled protein products were subjected to SDS/polyacrylamide-gel electrophoresis and autoradiography. Molecular mass standards are given for comparison in lanes 1 and 3 (from top to bottom): bovine serum albumin, 67 kDa; ovalbumin, 46 kDa; carbonic anhydrase, 30 kDa; lactoglobulin, 18.4 kDa; and cytochrome *c*, 12.3 kDa. Lanes 2 and 4 contain the translated peroxisomal CPT_s protein. This protein migrates just above the bovine serum albumin standard and calculates out to a molecular mass of 69.5 kDa.

mass of the CPT_s protein (from Western blots) derived from the insert was 65–75 kDa, similar to that of the mature peroxisomal CPT_s. Transcription assays were performed as described above, with the exception that the peroxisomal carnitine acyltransferase $pBlue_{ROX}$ clone RNA was used for the hybridization.

Materials

The amino acid sequence of the peroxisomal CPT_s tryptic fragment was generated by Dr. William Merrick, Department of Biochemistry, Case Western Reserve University, Cleveland, OH, U.S.A. *Eco*RI, *Hin*dIII and *Pst*I were purchased from BRL (Bethesda, MD, U.S.A.). pBluescript and T_7/T_3 mRNA capping kit were obtained from Stratagene (San Diego, CA, U.S.A.). Rabbit reticulocyte lysate translation and processing kits (endonuclease treated) and RNasin were obtained from Promega Biotech, Madison, WI, U.S.A. CoA was purchased from Pharmacia-PL Biochemicals (Piscataway, NJ, U.S.A.). Palmitoyl-CoA was synthesized according to Seubert (1960) using commercially available palmitoyl chloride (Sigma Chemical, St. Louis, MO, U.S.A.). Clofibrate and acetylsalicylic acid were from Sigma, and DEHP was from Aldrich Chemical Co., Milwaukee, WI, U.S.A. L-Carnitine was the kind gift of Sigma Tau, Rome, Italy. Ecolite biodegradable scintillation fluor was purchased from Westchem, San Diego, CA, U.S.A. [^{35}S]Methionine, [α-^{32}P]UTP, d[α-^{32}P]UTP and [γ-^{32}P]-ATP were obtained from DuPont–New England Nuclear, Boston, MA, U.S.A. Nitrocellulose was obtained from Schleicher & Schuell, Keene, NH, U.S.A. Immobilon-P was purchased from Enprotech, Hyde Park, MD, U.S.A. *Sequenase* was obtained from United States Biochemical, Cleveland, OH, U.S.A. All other chemicals were molecular biology grade, where available, or ACS

Table 1. Effect of various treatments on the activity, mRNA and transcription of CPT and CPT_s

Rats were fed diets containing the indicated ingredient of interest. Hepatic mitochondria and peroxisomes were isolated as described, and CPT or CPT_s activity was determined with palmitoyl-CoA or octanoyl-CoA as substrate. Immunoreactive protein (CPT) was determined by densitometric scans of Western blots. mRNA was determined by dot-blot or Northern blot hybridizations using cDNA clones labelled with ^{32}P and revealed by autoradiography. Transcription rates were determined by nuclear run-on assays and expressed relative to control values. The actual parts per million (ppm) were approx. 240 for CPT and 140 for CPT_s for the control rats. Values indicated by † have previously been published (Brady & Brady 1989*b*) and are presented for comparison only. Each value is the mean for the number of rats indicated in parentheses. The S.D. was derived from the error mean square of the analysis of variance. The level of statistical significance is indicated: *, $P < 0.05$; **, $P < 0.01$.

	Treatment						
	Control	Corn oil	Fish oil	Aspirin	Clofibrate	DEHP	S.D.
CPT:							
Activity (nmol/min per mg)	10.3 (5)	16.1 (3)	20.9 (3)	25.5† (5)	28.2† (5)	46.4 (5)	3.6**
Activity, relative	1.0 (5)	1.6 (3)	2.0 (3)	2.5† (5)	3.1† (5)	4.5 (5)	0.3**
Immunoreactive protein, relative	1.0 (5)	1.7 (3)	2.0 (2)	2.0† (5)	2.8† (5)	3.1 (5)	0.2**
mRNA, relative	1.0 (7)	1.7 (3)	3.2 (3)	1.9† (4)	2.7† (4)	4.3 (4)	1.1**
Transcription, relative	1.0 (7)	2.6 (3)	3.7 (3)	1.9† (4)	2.9† (4)	5.7 (4)	1.4**
CPT_s:							
Activity (nmol/min per mg)	4.6 (4)	11.6 (3)	12.5 (3)	7.8 (3)	11.0 (4)	16.6 (3)	4.0*
Activity, relative	1.0 (5)	1.6 (3)	2.0 (3)	1.7 (3)	2.4 (4)	3.6 (3)	0.9*
mRNA, relative	1.0 (4)	1.8 (3)	2.4 (3)	2.4 (4)	3.3 (4)	2.6 (4)	1.0*
Transcription, relative	1.0 (4)	10.4 (3)	13.7 (3)	2.4 (4)	3.3 (4)	4.4 (4)	2.0**

reagent grade. For most purposes, water was diethylpyrocarbonate-treated.

Statistics

Data were analysed by analysis of variance (ANOVA) for a multifactor design with unequal numbers using a general linear model with the PC-SAS system. Further post-analysis comparison of individual treatment means was done by Duncan's test using PC-SAS (Statistical Analysis Systems, Cary, NC, U.S.A.). Statistical significance is defined as $P < 0.05$.

RESULTS

Effect of fat on mitochondrial CPT and peroxisomal CPT_s

The activities of total mitochondrial CPT and the amount of immunoreactive CPT were increased 1.6–2-fold by consumption of either the corn oil or the fish oil diet compared with the control (Table 1). Western blots for the different groups showing the relative amount of immunoreactive protein are presented (Fig. 2*a*). The relative amounts of CPT mRNA and transcription rates were increased concurrently by 1.7–3-fold in rats fed high fish oil or corn oil diets compared with controls, (Table 1 and Northern blot, Fig. 3*a*). There were no differences in CPT activity, immunoreactive protein or mRNA levels when either corn oil or fish oil represented 25% of energy (kJ) (results not shown).

The activity of the peroxisomal CPT_s was increased by 2.5–3-fold by the consumption of high corn oil or fish oil diets compared with controls (Table 1). Immunoreactive protein was also increased with high fat diet consumption. Western blots showing the relative immunoreactive protein for the groups are presented in Fig. 2(*b*). The total amount of mRNA for peroxisomal CPT_s was increased by 1.8-fold by corn-oil feeding and 2.4-fold by fish-oil feeding, while transcription rate was increased by 10.4- and 13.7-fold by corn oil and fish oil respectively (Table 1 and Northern blot, Fig. 3*b*).

Effect of drugs on mitochondrial CPT and peroxisomal CPT_s

Data for the effect of acetylsalicylic acid and clofibrate on CPT mRNA and transcription have been presented (Brady & Brady, 1989*b*). These data showed 2–3-fold increases in mitochondrial CPT activity, immunoreactive

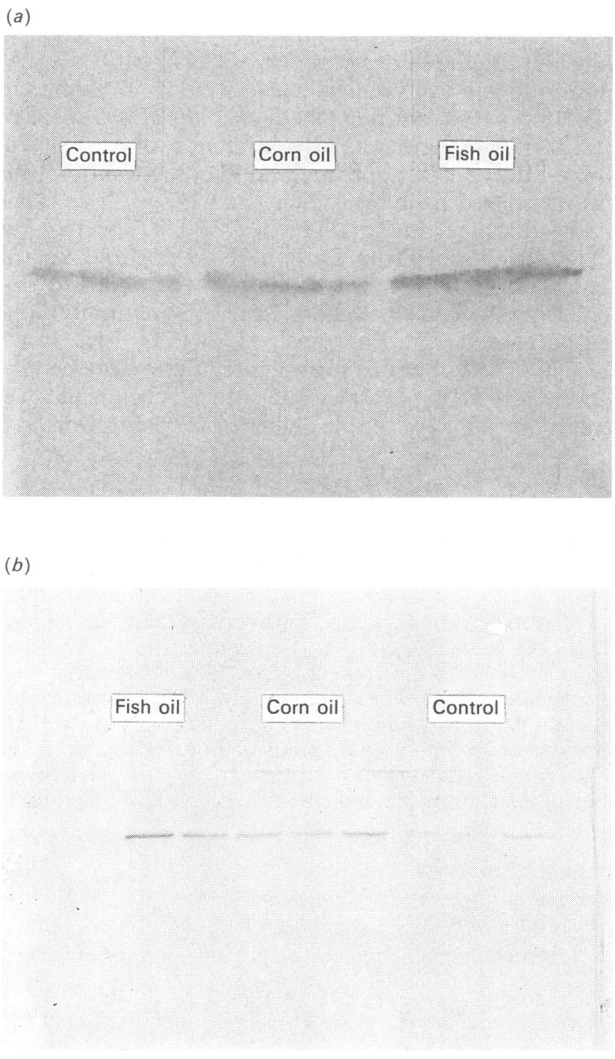

Fig. 2. Western blot of hepatic (*a*) CPT and (*b*) CPT_s

Approx. 1 mg of mitochondrial or peroxisomal protein from samples each of control, corn-oil or fish-oil fed rats was applied to a 1.5 mm SDS/polyacrylamide-gel electrophoresis gel, transferred to Immobilon and revealed using rabbit anti-CPT or anti-CPT_s, and mouse anti-rabbit IgG linked to alkaline phosphatase.

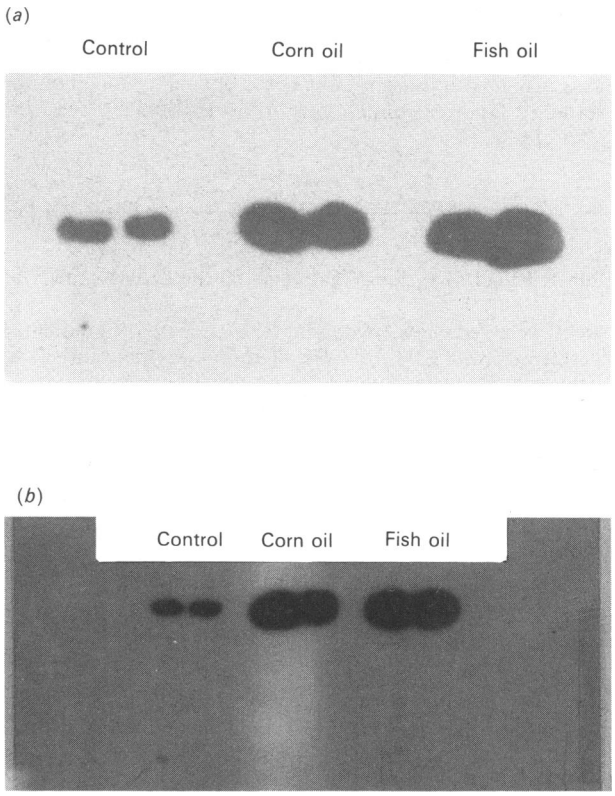

Fig. 3. Northern blots for hepatic (*a*) CPT and (*b*) CPT_s

Approx. 20 μg of total hepatic RNA was applied to a 1.2% agarose–formaldehyde gel, transferred to nitrocellulose and revealed with ^{32}P-labelled specific probes (described under Materials and methods) prior to autoradiography.

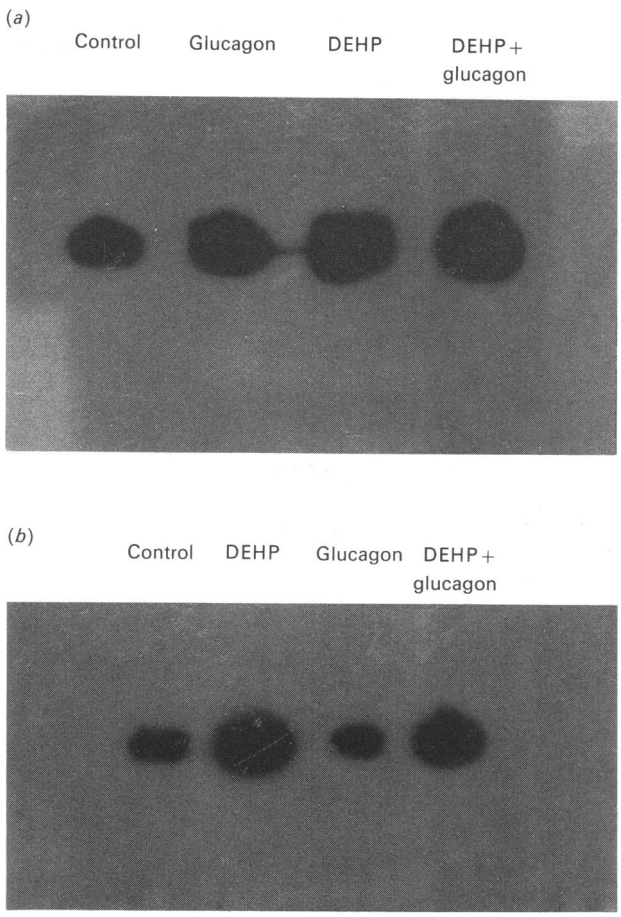

Fig. 4. Northern blots for hepatic RNA for (*a*) CPT and (*b*) CPT_s of DEHP-fed rats

Northern blots were generated as described in Fig. 3, the Materials and methods section, and Brady *et al.* (1988).

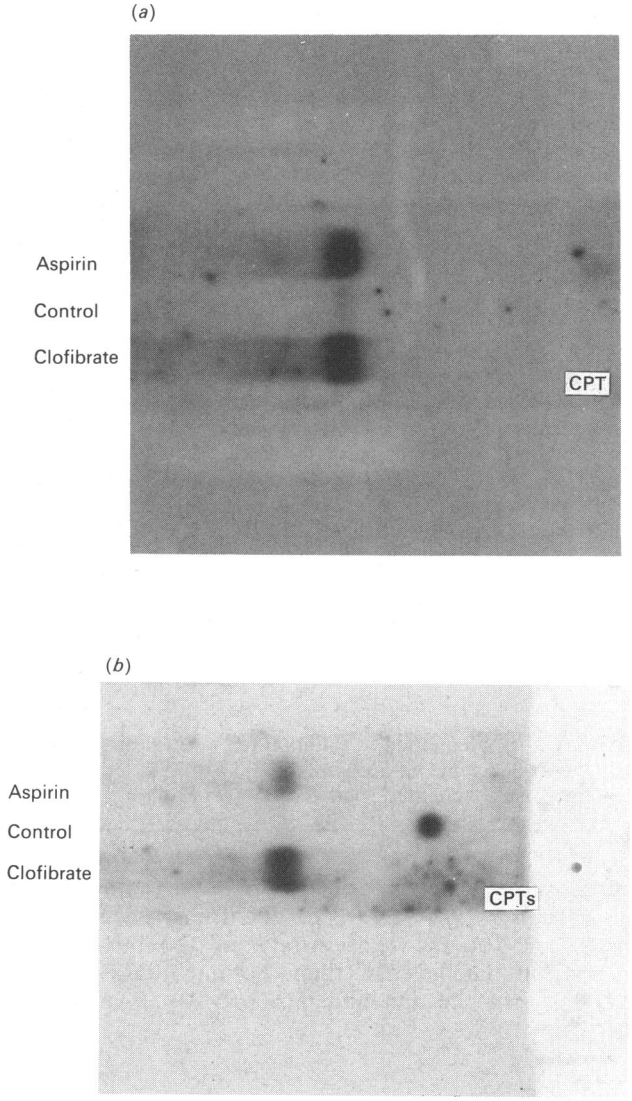

Fig. 5. Northern blots of hepatic RNA for (*a*) CPT and (*b*) CPT_s using aspirin- and clofibrate-treated rats

Northern blots were generated as described in Fig. 3 and in Brady *et al.* (1988). The drug administration to the rats was as described in the Materials and methods section.

protein, mRNA and transcription rates for CPT when clofibrate or acetylsalicylic acid was fed to rats (see also Fig. 5*a*). When DEHP was fed, the increase in CPT activity was 4.5-fold; for immunoreactive CPT, 3.1-fold; for CPT mRNA, 4.5-fold; and for transcription rate of CPT RNA, 5.7-fold (Table 1 and Fig. 4*a*). Glucagon increased both CPT mRNA (Fig. 4*a*) and transcription rate by 3-fold (results not shown), but did not increase CPT activity or immunoreactive CPT (not shown) by 5 h. The data for the peroxisomal CPT_s were very similar. Peroxisomal CPT_s activity was increased by 2.4-fold with clofibrate, 1.7-fold with acetylsalicylic acid and 3.6-fold with DEHP feeding (Table 1). Northern blots are presented to document that mRNA for CPT_s is increased by DEHP (Fig. 4*b*), clofibrate and acetylsalicylic acid (Fig. 5*b*). Dot-blot analysis showed that mRNA for CPT_s was increased by 2.4-fold by acetylsalicylic acid; 3.3-fold by clofibrate and 2.6-fold by DEHP. Glucagon did not increase peroxisomal CPT_s mRNA (Fig. 4*b*). Transcription rates for CPT_s were increased by 2.4-, 3.3- and 4.4-fold for acetylsalicylic acid, clofibrate and DEHP respectively. We also examined the effects of riboflavin deficiency (decreased FAD cofactor leads to decreased activity of acyl-CoA dehydrogenase and depression of β-oxidation in mitochondria) on CPT_s. We had previously determined that CPT mRNA was increased by 3–5-fold with riboflavin deficiency and starvation (Brady *et al.*, 1988). Peroxisomal CPT_s mRNA was increased 6-fold after 48 h starvation and 3.3-fold in riboflavin deficiency, but was not significantly affected by the pair-feeding regimen (Table 2).

Correlations between the mRNA and transcription rates for mitochondrial CPT and peroxisomal CPT_s are presented in Fig. 6. When all data from all treatments were combined, the correlation for CPT mRNA and CPT_s mRNA was significant (correlation coefficient of 0.6); however, this did not hold true for the transcription rates. This disparity was due to a larger increase in transcription rate of CPT_s for animals fed corn oil and fish oil than those fed acetylsalicylic acid, clofibrate or DEHP. When the diet effects were separated from the drug effects and two lines generated, the correlation coefficients for the relationship between CPT and CPT_s

Table 2. Effect of riboflavin deficiency and starvation on CPT_s mRNA

Hepatic RNA was isolated from rats fed one of three dietary regimens for four weeks as described previously (Brady *et al.*, 1988). Riboflavin supplemented, *ad libitum*-fed rats were given the riboflavin-supplemented diet *ad libitum*. Riboflavin-deficient rats were fed the same diet formulation without riboflavin. Riboflavin-supplemented, pair-fed rats were fed the riboflavin-supplemented diet at a level equivalent to the food consumption of the riboflavin-deficient animals. RNA was quantified using a ^{32}P-labelled 17-mer probe to hybridize to 5 and 10 μg of total RNA on nitrocellulose. RNA was quantified by counting the dots. Values are expressed as the multiple of the mean control value (riboflavin supplemented, fed). Each value is the mean of three rats. The S.E.M., derived from the error mean square of the analysis of variance, was 0.7 [Significant effects: diet ($P < 0.01$), starvation ($P < 0.01$)]. Individual differences were tested by Duncan's test. Means with different letter superscripts are different ($P < 0.05$).

	Treatment	
Diet	Fed	Starved, 48 h
Riboflavin-supplemented, *ad libitum*-fed	1.0^{a}	6.0^{c}
Riboflavin-deficient, *ad libitum*-fed	3.3^{b}	3.7^{bc}
Riboflavin-supplemented, pair-fed	1.5^{ab}	2.8^{ab}

transcription were 0.80 ($n = 13$, $P < 0.01$) for the diet and 0.79 ($n = 19$, $P < 0.01$) for the drugs. The same was then done for the relationships of the mRNA; the correlation coefficients then became 0.61 ($n = 13$; $P < 0.05$) for the diet and 0.69 ($n = 19$, $P < 0.01$) for the drugs.

DISCUSSION

The present data confirm that both mitochondrial CPT (total activity) and peroxisomal CPT_s are generally induced concomitantly by either drugs or diets that induce peroxisomal fatty acid metabolism. When β-oxidation was depressed in riboflavin deficiency (where acyl CoA dehydrogenase activity is depressed), the mRNAs for CPT and for CPT_s were increased significantly (present data and Brady *et al.*, 1988).

Consumption of diets high in corn oil or fish oil by rats for 4 weeks led to increased activity, immunoreactive protein, mRNA levels and transcription rates for mitochondrial CPT and peroxisomal CPT_s in liver. These results support data in the literature that consumption of a high fat diet increases peroxisomal and mitochondrial β-oxidation and amounts of enzymes, but do not support a specific role for fish oil in increasing the activity and synthesis of mitochondrial CPT or the peroxisomal CPT_s. These data, and the data showing that the synthesis of both enzymes is induced concurrently by DEHP, clofibrate and acetylsalicylic acid, further support the studies in the literature that suggest co-ordinate induction of both β-oxidation and the synthesis of enzymes of β-oxidation in peroxisomes and mitochondria.

Previous studies comparing fish oil with other animal fats or with polyunsaturated oils from plants concluded that synthesis of triglycerides was specifically depressed by fish oils to a greater extent than by unsaturated plant oils or animal fat (Iritani *et al.*, 1982; Marsh *et al.*, 1987; Strum-Odin *et al.*, 1987). Other studies have found that β-oxidative enzyme activities and rates of fatty acid

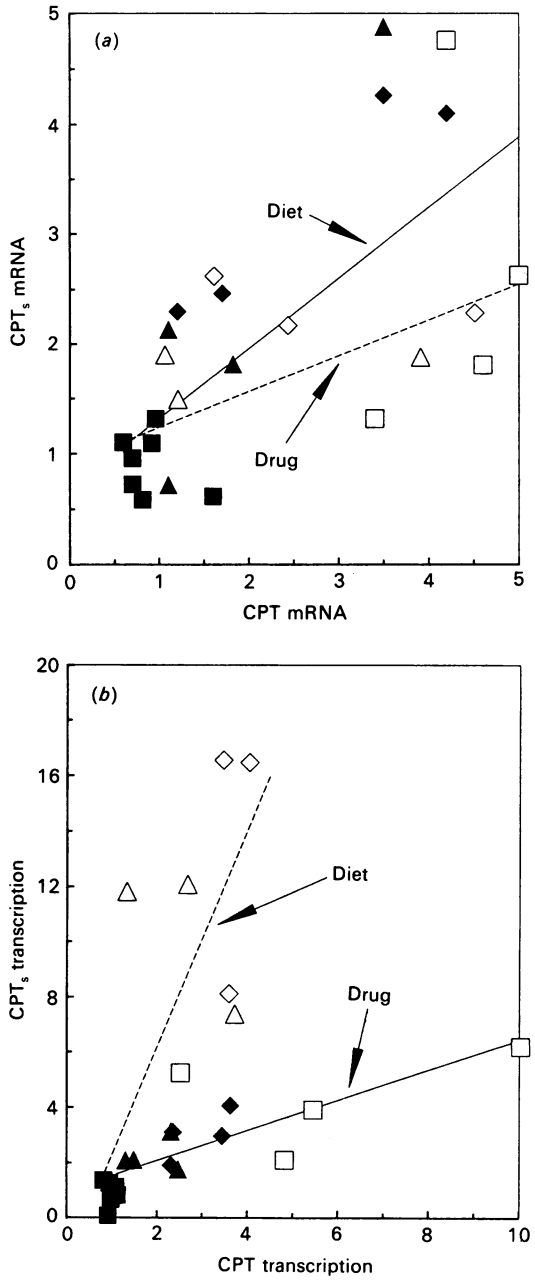

Fig. 6. Correlations for CPT and CPT_s for (*a*) mRNA and (*b*) transcription under various dietary and pharmacological treatments

(*a*) Correlation of hepatic CPT and CPT_s mRNA levels in rats under various dietary and pharmacological treatments. ■, control; ◇, high fat diet; ◆, clofibrate feeding; □, DEHP-feeding; ▲, acetylsalicylic acid feeding. The linear regression line designated 'Diet' was derived using data for dietary treatments and the line designated 'Drug' was derived using data for drug treatments of rats. (*b*) Correlation of CPT and CPT_s transcription under various dietary and pharmacological treatments; details the same as for (*a*). All values are relative to controls.

oxidation were increased to a greater extent by fish oil than by polyunsaturated plant oils (Neat *et al.*, 1980; Thomassen *et al.*, 1982; Berge *et al.*, 1987). Berge *et al.* (1987) found that CPT activity (total and outer) increased when partially hydrogenated marine oils were fed without added essential fatty acids, but that the increase was diminished when linoleic acid was added back. In the present experiments, essential fatty acids were present in both high fat diets, as basal amounts of corn oil were added to the fish oil diet. Mitochondrial CPT activity and synthesis were similar for rats fed both corn oil and fish oil. The percentage of kJ obtained from fat was the major factor in the increase in CPT and CPT_s, since there was no effect on CPT activity or synthesis when either corn oil or fish oil represented 25% of dietary energy. Our conclusion is that a high fat diet is a major inducer of total mitochondrial CPT activity and CPT synthesis. The same appears to be true of the peroxisomal CPT_s.

Peroxisomal and mitochondrial β-oxidation and enzyme activities have also been found to increase in tandem when clofibrate or other hypolipidaemic agents are administered (Markwell *et al.*, 1977; Mannaerts *et al.*, 1979; Ganning & Dallner, 1981; Silcox *et al.*, 1982; Farrell & Bieber, 1983; Ozasa *et al.*, 1983; Veerkamp & van Moerkerk, 1986; Henninger *et al.*, 1987). However, the magnitude of the increases in peroxisomal versus mitochondrial β-oxidation and enzyme activity are not always equivalent. Mannaerts *et al.* (1979) found that clofibrate induced β-oxidation and enzymes in peroxisomes to a greater extent than in mitochondria. Farrell & Bieber (1983) found that, when hypolipidaemic drugs were fed, the specific activity of mitochondrial acyltransferases increased to a greater extent than that of peroxisomal acyltransferases. Reddy *et al.* (1986) have also shown that peroxisomal enzymes' transcription and mRNA levels can increase rapidly in response to peroxisome-proliferating drugs.

We had previously found that starvation and riboflavin deficiency (which depresses mitochondrial acyl-CoA dehydrogenase activity) increased the synthesis of mitochondrial CPT (Brady *et al.*, 1988). In the present study we extend these observations to peroxisomal CPT_s. The mRNA for the peroxisomal CPT_s was increased 3.3-fold by riboflavin deficiency and 6-fold by 48 h starvation. Preliminary experiments using pentenoic acid, which also inhibits β-oxidation at a step other than CPT, show that mitochondrial CPT mRNA is increased 2-fold, while peroxisomal CPT_s mRNA is not affected (P. S. Brady & L. J. Brady, unpublished work). These experiments indicate that intra-mitochondrial build-up of acyl-CoAs may produce a signal for increased mitochondrial CPT synthesis, although the mechanism cannot be discerned at this point.

Neither the general mechanism nor separate specific mechanisms which control nutritional and pharmacological induction of these enzymes concomitantly can be anything but speculative at this point. It appears that there is a difference in the mechanism of induction of CPT_s when diet versus drugs are used as inducing agents (transcription rates were induced over 10-fold by high fat, but only 2–4-fold by drugs). Thus, it is possible that diet may act by hormonal changes, while drugs act as inducing agents via another mechanism. However, one would not necessarily draw this conclusion from the CPT induction data, where diet and drugs had effects of similar magnitude on CPT synthesis.

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