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Carnitine palmitoyltransferase in liver and five extrahepatic tissues in the rat

Inhibition by DL-2-bromopalmitoyl-CoA and effect of hypothyroidism

E. David SAGGERSON and Carol A. CARPENTER
Department of Biochemistry, University College London, Gower Street, London WC1E 6BT, U.K.

1. Mitochondria were isolated from rat adult liver, foetal liver, kidney cortex, heart, skeletal muscle and interscapular brown adipose tissue. 2 DL-2-Bromopalmitoyl-CoA inhibited the overt form of carnitine palmitoyltransferase (CPT_1) in heart, skeletal muscle and brown adipose tissue, with an IC_{50} value (concentration giving 50% inhibition) of 1.3–1.6 μM. By contrast, the IC_{50} value for inhibition of the kidney or adult liver enzyme was 0.08–0.1 μM. 3. CPT_1 in near-term foetal liver differed from that in adult liver in that the IC_{50} for inhibition by 2-bromopalmitoyl-CoA was 0.57 μM. 4. It is suggested that there may be tissue-specific forms of the catalytic entity of CPT_1 and that foetal liver may contain a mixture of adult liver- and muscle-type enzymes. 5. In rats made hypothyroid by administration of propylthiouracil and an iodine-deficient diet, hepatic CPT_1 activity was decreased by 83%. However, CPT_1 activity in extrahepatic tissues showed no adaptive decrease in hypothyroidism.

INTRODUCTION

The overt form of carnitine palmitoyltransferase (CPT_1) appears to play an important role in regulating the flux of long-chain fatty acids into mitochondrial oxidative metabolism in mammalian tissues. The purpose of the present study was to examine certain aspects of differences in the enzyme or in its regulation between various tissues.

Previous studies have revealed some marked differences in kinetic properties between rat liver CPT_1 and the enzyme in certain extrahepatic tissues. For example, the liver and heart enzymes differ in their response to changes in $[K^+]$ and $[Mg^{2+}]$ (Saggerson, 1982); the K_m for carnitine of the skeletal-muscle or heart enzyme is substantially higher than that of the liver CPT_1 (McGarry *et al.*, 1983; Mills *et al.*, 1984), and CPT_1 in brown adipose tissue, heart or skeletal-muscle mitochondria is far more sensitive to inhibition by malonyl-CoA than is the enzyme from liver and kidney cortex (Saggerson & Carpenter, 1981*b*, 1982*a*; McGarry *et al.*, 1983; Mills *et al.*, 1983, 1984). It is possible that this latter difference in properties is due to inert-tissue variation in the characteristics of a malonyl-CoA-binding site that is distinct from the catalytic entity of the CPT_1 (McGarry, *et al.*, 1978; Saggerson, 1982; Mills *et al.*, 1984; Bird & Saggerson, 1984; Edwards *et al.*, 1985; Bremer *et al.*, 1985). We (Edwards *et al.*, 1985) compared the effects of the inhibitor 2-bromopalmitoyl-CoA on the enzyme in rat liver and heart mitochondria, and surprisingly, found that the inhibitory potency was 17 times greater with liver than with heart CPT_1. Since it is likely that this inhibition is at the catalytic site, this finding suggests the likelihood of substantial inter-tissue differences in the catalytic entity of the enzyme. Accordingly, we have investigated the effect of this inhibitor on CPT_1 from several rat tissues.

It is also likely that there are inter-tissue differences in the adaptive response of CPT_1 activity to alteration of physiological state. The liver specific activity changes with nutritional and hormonal status, increasing in starvation (Harper & Saggerson, 1975; Bremer, 1981; Bird & Saggerson, 1984; Saggerson *et al.*, 1984; Cook, 1984), in diabetes (Harano *et al.*, 1972) and hyperthyroidism (Stakkestad & Bremer, 1983), and decreasing in hypothyroidism (Saggerson *et al.*, 1982; Stakkestad & Bremer, 1983; Bird & Saggerson, 1984). In fact, the extent of the change in liver CPT_1 activity in hypothyroidism is only matched in scale by the substantial increase in hepatic activity that occurs at birth (Augenfeld & Fritz, 1970; Foster & Bailey, 1976; Yeh & Zee, 1979; Saggerson & Carpenter, 1982*b*). Changes in specific activity of the hepatic enzyme with physiological states are often accompanied by changes in sensitivity to the inhibitor malonyl-CoA (Saggerson & Carpenter, 1981*a*,*c*, 1982*b*; Bremer, 1981; Robinson & Zammit, 1982; Stakkestad & Bremer, 1983; Cook, 1984; Cook *et al.*, 1984; Gamble & Cook, 1985). Adaptations in specific activity or of malonyl-CoA sensitivity have not been extensively investigated in extrahepatic tissues, but, where examined, extrahepatic CPT_1 appears to be far more constitutive. Heart, skeletal-muscle and white-adipose-tissue CPT_1 show little or no change in specific activity or malonyl-CoA sensitivity in starvation (Harper & Saggerson, 1975; Veerkamp & Van Moerkerk, 1982; Saggerson & Carpenter, 1983; Paulson *et al.*, 1984; Cook, 1984). In view of the profound effects that hypothyroidism has on whole-body metabolism, and in view of the large change that this state brings about in hepatic CPT_1 activity, we decided to investigate whether hypothyroidism brings

Abbreviations used: CPT_1, the overt form of carnitine palmitoyltransferase (EC 2.3.1.21); IC_{50}, the concentration of inhibitor causing 50% inhibition of CPT_1.

about equivalent alterations in the activity of the enzyme in various extrahepatic tissues.

MATERIALS AND METHODS

Animals

These were male Sprague–Dawley rats bred at University College London. All animals had constant access to drinking water and to Rat and Mouse No. 3 Breeding Diet (Special Diet Services, Witham, Essex, U.K.), which contained (w/w) 21% digestible crude protein, 4% digestible crude oil and 39% starches and sugars. The light/dark cycle was 13 h/11 h, with light from 06:00 to 19:00 h. In studies with 2-bromopalmitoyl-CoA, animals were aged 6 weeks (160–180 g body wt.). For study of hypothyroidism 15 animals (116±1 g body wt.) were selected at age 4 weeks and then fed on an iodine-deficient version of the No. 3 Breeding Diet and drank water containing 0.01% (w/v) 6-n-propyl-2-thiouracil. Ethanol was used initially to dissolve the propylthiouracil and hence was also present in the drinking water at 0.25% (v/v). Hypothyroid rats were killed 27–38 days after commencement of this treatment, when they weighed 182±5 g and had exhibited negligible growth after day 21 of treatment. This procedure causes severe depression of the plasma concentrations of thyroid hormones (Chohan *et al.*, 1984). Ten euthyroid controls were also selected at age 4 weeks (body wt. 113±2 g) and maintained for 28–30 days on the No. 3 Diet with 0.25% ethanol in the drinking water. These animals grew steadily and weighed 306±8 g at death.

Chemicals

Sources of these were as described by Saggerson *et al.* (1982), Chohan *et al.* (1984) and Edwards *et al.* (1985). DL-2-Bromopalmitoyl-CoA was synthesized by Dr. M. I. Bird from 2-bromopalmitic acid via 2-bromopalmitoyl chloride as described by Seubert (1960) and Chase & Tubbs (1972). The product was assayed enzymically by measuring the release of CoA catalysed by rat brain palmitoyl-CoA hydrolase (EC 3.1.2.2), which was partially purified by Dr. M. R. Edwards by the method of Anderson & Erwin (1971). The CoA release was measured with 5,5′-dithiobis-(2-nitrobenzoic acid) (Knauer, 1979). The 2-bromopalmitoyl-CoA contained negligible free thiol and was stored as a 3mM aqueous solution at −40 °C.

Isolation of mitochondria

Mitochondria were obtained from heart and adult liver as described by Saggerson (1982), from interscapular brown adipose tissue as described by Saggerson & Carpenter (1982*a*) and from kidney cortex and skeletal muscle as described by Saggerson & Carpenter (1981*b*). Foetal liver mitochondria were prepared from the pooled livers of one litter (10–12 foetuses) by the same method as for adult liver (Saggerson, 1982). These were obtained at 20–21 days gestation. In all cases mitochondria were finally suspended in ice-cold 0.3 M-sucrose medium containing 10 mM-Tris/HCl buffer (pH 7.4) and 1 mM-EGTA and used for CPT_1 assays within 1 h. Mitochondrial protein was measured by the method of Lowry *et al.* (1951), with bovine serum albumin as standard.

Assay of CPT_1

Portions (50 μl) of the stock mitochondrial suspensions were used. These contained the following average amounts of mitochondrial protein (mg); adult liver, 0.24; foetal liver, 0.36; heart, 0.14; skeletal muscle, 0.20; kidney cortex, 0.23; brown adipose tissue, 0.12. All assays were at 25 °C in 1.0 ml volumes containing 25 mM-Tris/HCl buffer (pH 7.4), 150 mM-sucrose, 60 mM-KCl, fatty acid-poor albumin (1.3 mg/ml) and 1 mM-EDTA. For comparison of CPT_1 activities in the euthyroid and hypothyroid states, the mitochondria were preincubated in this medium together with 1 mM-dithiothreitol and 100 mM-palmitoyl-CoA for 2 min. The reaction was then initiated by addition of 20 μl containing 0.4 μmol of L-carnitine and 0.5 μCi of L-[*Me*-^{3}H]carnitine. After 4 min. the reaction was terminated, and incorporation of ^{3}H into butan-1-ol-soluble products determined (Saggerson *et al.*, 1982). For investigation of the effects of 2-bromopalmitoyl-CoA, dithiothreitol was omitted because of its reactivity with this compound. In these assays mitochondria were preincubated for 2 min in the Tris/sucrose/KCl/albumin/EGTA medium together with the required concentration of 2-bromopalmitoyl-CoA, 0.4 mM-L-carnitine and 0.5 μCi of L-[*Me*-^{3}H]carnitine. The reaction was then initiated by addition of 100 μM-palmitoyl-CoA with rapid mixing. The reaction was determined after 4 min and treated as above.

Assay of citrate synthase (EC 4.1.3.7)

This was performed spectrophotometrically at 25 °C and 412 nm in 1.0 ml containing 100 mM-Tris/HCl buffer (pH 8.0), 0.1% (w/v) Triton X-100, 0.1 mM-5,5′-dithiobis-(2-nitrobenzoic acid), 50 μM-acetyl-CoA and 20 μl of mitochondria (diluted 1 in 5 for heart, skeletal muscle and brown adipose tissue). The reaction was initiated by addition of 50 nmol of oxaloacetate.

Statistical methods and presentation of data

Throughout, values are shown as means±S.E.M. Where S.E.M. bars are not shown in Figures, these lie within the area of the symbol. Statistical significance was determined by Student's *t* test for unpaired samples. Values of *n* in legends refer to the number of separate mitochondrial preparations.

RESULTS AND DISCUSSION

Effect of DL-2-bromopalmitoyl-CoA

In these particular experiments CPT_1 assays were performed differently from those of Edwards *et al.* (1985). Firstly, mitochondria were preincubated with 2-bromopalmitoyl-CoA+[^{3}H]carnitine before initiation of the reaction with palmitoyl-CoA. In preliminary experiments it was found that this approach gave IC_{50} values for inhibition of the adult liver or heart enzyme that were approx. 3-fold lower than those obtained when the order of addition of [^{3}H]carnitine and palmitoyl-CoA was reversed. These was advantageous, since appreciable inhibition of heart CPT_1 could then be achieved with 0.1–10 μM-2-bromopalmitoyl-CoA (compare with Fig. 1 of Edwards *et al.*, 1985). Secondly, the concentration of palmitoyl-CoA was 100 μM rather than 40 μM. This alteration was desirable because, with albumin present at 1.3 mg/ml, 100 μM-palmitoyl-CoA gives $V_{max.}$ conditions with respect to this substrate with adult liver (Saggerson

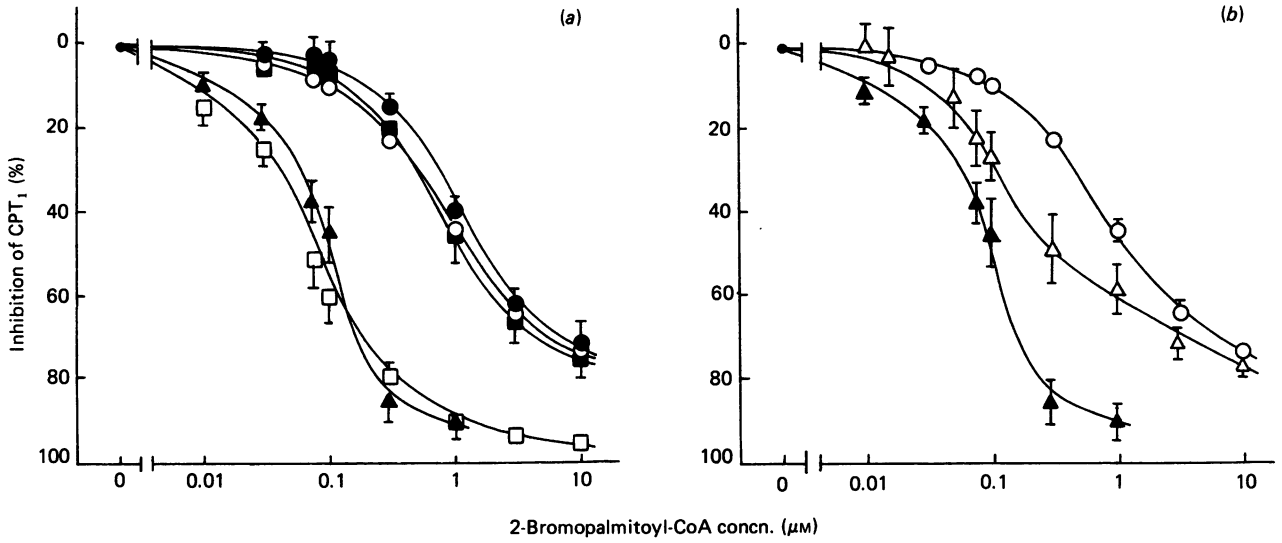

Fig. 1. Inhibition of CPT_1 by DL-2-bromopalmitoyl-CoA

Symbols and CPT_1 activities in the absence of the inhibitor (expressed as nmol/min per mg of protein) are as follows: ○, heart, 15.5 ± 0.6 ($n = 3$); ●, brown adipose tissue, 17.9 ± 1.7 ($n = 3$); □, kidney cortex, 3.5 ± 0.3 ($n = 3$); ■, skeletal muscle, 7.9 ± 0.3 ($n = 3$); △, foetal liver, 0.96 ± 0.11 ($n = 6$); ▲, adult liver, 5.2 ± 0.2 ($n = 3$).

Table 1. Inter-tissue differences in IC_{50} values for inhibition of CPT_1 by DL-2-bromopalmitoyl-CoA and malonyl-CoA

IC_{50} values for 2-bromopalmitoyl-CoA are derived from the experiments shown in Fig. 1. IC_{50} values for malonyl-CoA are calculated from data in previous publications from this laboratory as shown. The numbers in parentheses indicate the number of separate values. Significance of difference of values from those for adult liver is indicated as follows: [a]non-significant; [b]$P < 0.05$; [c]$P < 0.02$; [d]$P < 0.01$; [e]$P < 0.001$.

Tissue	IC_{50} for 2-bromopalmitoyl-CoA (μM)	IC_{50} for malonyl-CoA (μM)	
Heart	1.32 ± 0.14^{d} (3)	0.10 ± 0.01^{e} (3)	(Saggerson & Carpenter, 1981*b*)
Skeletal muscle	1.30 ± 0.30^{c} (3)	0.14 ± 0.01^{e} (3)	(Saggerson & Carpenter, 1981*b*)
Brown adipose tissue	1.61 ± 0.32^{d} (3)	0.24 ± 0.06^{e} (3)	Saggerson & Carpenter, 1982*a*)
Kidney cortex	0.085 ± 0.015^{a} (3)	8.2 ± 1.1^{a} (3)	(Saggerson & Carpenter, 1981*b*)
Adult liver	0.11 ± 0.019 (3)	12.3 ± 2.4 (3)	(Saggerson & Carpenter, 1981*a*; Saggerson *et al.*, 1982)
Foetal liver	0.57 ± 0.18^{b} (6)	0.48 ± 0.04^{e} (4)	(Saggerson & Carpenter, 1982*b*)

& Carpenter, 1981*c*), foetal liver (Saggerson & Carpenter, 1982*b*), heart (Edwards *et al.*, 1985), brown adipose tissue (Saggerson & Carpenter, 1982*a*) and kidney cortex and skeletal muscle (preliminary experiments; results not shown). Thus comparisons of IC_{50} values between the tissues are made under comparable conditions. Fig. 1 and Table 1 show that heart, skeletal-muscle and brown-adipose-tissue activities all had very similar sensitivity to 2-bromopalmitoyl-CoA. Likewise the IC_{50} values for adult liver and kidney were very similar to each other, but were approx. 15-fold less than those for the former three tissues. Clearly, in tissues where CPT_1 is very sensitive to malonyl-CoA the enzyme is relatively insensitive to the catalytic-site-directed inhibitor, and vice versa in adult liver and kidney cortex. Bird & Saggerson (1984), Mills *et al.* (1984) and Edwards *et al.* (1985) have discussed the likelihood that the malonyl-CoA-binding site and the catalytic site of CPT_1 are distinct. Furthermore, since sensitivity to malonyl-CoA is lost on solubilization of the membrane with detergents (McGarry *et al.*, 1978; Saggerson, 1982) or on purification of the enzyme (Fiol & Bieber, 1984), it would seem probable that these distinct sites are on separate polypeptides. The present results suggest that there are distinct tissue-specific forms of both of these entities. Whether there is a tissue-specific form of each that is common to liver and kidney cortex and a separate part that is common to heart, skeletal muscle and brown adipose tissue, or whether the situation is

more complex, will not be resolved until the components can be compared immunologically. Nevertheless one is struck by the closeness with which these adult tissues fall into two distinct 'families'.

Near to term, rat foetal liver mitochondria contain a low CPT_1 activity that is considerably more sensitive to malonyl-CoA than is the liver enzyme of adult rats (Saggerson & Carpenter, 1982*b*). Fig. 1(*b*) and Table 1 show that foetal liver contains CPT_1 with sensitivity to 2-bromopalmitoyl-CoA that is intermediate between that of the two adult 'families'. Such a curve can be generated from a mixture where approx. 40% of the CPT_1 activity has an IC_{50} for 2-bromopalmitoyl-CoA of 0.1 μM and the remaining 60% has an IC_{50} of 1.3 μM. Similarly it can be calculated that a 60%/40% mixture of CPT_1 activities with IC_{50} values for malonyl-CoA of 0.1 μM and 10 μM respectively would be 50% inhibited by approx. 0.5 μM-malonyl-CoA. At birth, rat liver CPT_1 activity is increased severalfold (Augenfeld & Fritz, 1970; Foster & Bailey, 1976; Yeh & Zee, 1979) and the enzyme assumes a lower sensitivity to malonyl-CoA (Saggerson & Carpenter, 1982*b*). It is suggested that at term the liver contains a CPT_1 that is 'muscle-type' and that this is in the process of being superseded by the adult liver forms of the catalytic entity and the malonyl-CoA-binding component.

Effect of hypothyroidism

Subjecting rats to a combination of an iodine-deficient diet together with propylthiouracil in the drinking water for at least 4 weeks resulted in an 83% decrease in the specific activity of CPT_1 in liver mitochondria (Table 2). This may be contrasted with a decrease of 55% when rats are treated with propylthiouracil alone (Stakkestad & Bremer, 1983) or are thyroidectomized (Saggerson *et al.*, 1982). However, CPT_1 activity was not decreased by hypothyroidism in any of the other four tissues examined, and in fact the specific activity of the kidney enzyme was increased by 25% and that of the skeletal-muscle enzyme apparently was doubled. It was considered that possibly the euthyroid and hypothyroid states might differ in the amount of non-mitochondrial protein contaminating the mitochondrial fractions. For this reason, citrate synthase activity was also measured as a reference activity characteristic of the mitochondrial fraction. As found by Saggerson *et al.* (1982), hypothyroidism decreased the specific activity of citrate synthase in liver mitochondria. On the other hand, there was little change in citrate synthase activity in mitochondria from the extrahepatic tissues. Hence the modest increases in CPT_1 activity in kidney cortex and skeletal muscle are still observed when this activity is expressed relative to citrate synthase.

Hypothyroidism diminishes the fatty acid oxidation that occurs on electrical or hormonal stimulation of brown adipose tissue (Seydoux *et al.*, 1982; Sundin *et al.*, 1984). The present results suggest that adaptation in CPT_1 activity is unlikely to be the cause of this lesion.

It was noted that the specific activity of CPT_1 in skeletal-muscle mitochondria from the 9-week-old euthyroid rats (Table 2) was substantially lower than in mitochondria from the 6-week-old animals used in the study with 2-bromopalmitoyl-CoA (see the legend to Fig. 1). An additional experiment was therefore performed in which skeletal-muscle mitochondria were prepared simultaneously from three euthyroid rats of body wt. 168 ± 3 g and from three older rats of body wt. 299 ± 4 g. CPT_1 specific activities in these mitochondria were 8.5 ± 0.5 and 5.4 ± 0.7 nmol/min per mg of protein for the smaller and the larger rats respectively ($P < 0.05$). Thus an age- or size-related decrease in the specific activity of muscle CPT_1 is normally seen, and it would appear that this is abolished in hypothyroidism rather than that hypothyroid actually increases the activity.

In conclusion, CPT_1 activity in these extrahepatic tissues does not shown the downward adaptivity that is seen in liver in hypothyroidism. These hypothyroid rats have a severe impairment of adipose-tissue lipolysis (results not shown), and lowered plasma concentrations of non-esterified fatty acids are seen in hypothyroidism (Nikkila & Kekki, 1972). The retention of CPT_1 in the hypothyroid state in these tissues will ensure that their oxidative metabolism retains a share of the diminished availability of fatty acids.

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Table 2. CPT_1 and citrate synthase activities in mitochondria from rat tissues in the euthyroid (E) and hypothyroid (H) states

$^aP < 0.02$, $^bP < 0.01$, $^cP < 0.001$ respectively for comparison of the euthyroid and hypothyroid states; $n = 4$ for all values.

Tissue	Condition	Citrate synthase activity (nmol/min per mg of protein)	CPT_1 (nmol/min per mg of protein)	$10^3 \times \frac{CPT_1 \text{ activity}}{\text{citrate synthase activity}}$
Liver	E	120 ± 2	4.2 ± 0.4	35.2 ± 3.8
	H	86 ± 3^c	0.7 ± 0.1^c	8.2 ± 1.6^c
Heart	E	1073 ± 44	19.3 ± 0.5	18.0 ± 0.3
	H	1116 ± 23	20.8 ± 1.1	18.7 ± 1.2
Brown fat	E	1177 ± 63	11.4 ± 0.9	9.7 ± 0.4
	H	1393 ± 157	11.5 ± 0.9	8.4 ± 0.5
Kidney cortex	E	231 ± 3	3.9 ± 0.2	16.8 ± 0.9
	H	205 ± 6^b	4.9 ± 0.2^a	23.8 ± 0.8^b
Skeletal muscle	E	238 ± 18	3.3 ± 0.1	14.1 ± 0.6
	H	328 ± 38	6.6 ± 0.9^a	19.8 ± 1.0^b

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