Effects of thyroidectomy and starvation on the activity and properties of hepatic carnitine palmitoyltransferase

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1. Hepatic carnitine palmitoyltransferase activity was measured over a range of concentrations of palmitoyl-CoA and in the presence of several concentrations of the inhibitor malonyl-CoA. These measurements were made in mitochondria obtained from the livers of fed and starved (24 h) normal rats and of fed and starved thyroidectomized rats. 2. In the fed state thyroidectomy substantially decreased overt carnitine palmitoyltransferase activity and also decreased both the Hill coefficient and the $s_{0.5}$ when palmitoyl-CoA concentration was varied as substrate. Thyroidectomy did not appreciably alter the inhibitory effect of malonyl-CoA on the enzyme. 3. Starvation increased overt carnitine palmitoyltransferase activity in both the fed and the thyroidectomized state. In percentage terms this response to starvation was substantially greater after thyroidectomy. In both the hypothyroid and normal states starvation decreased sensitivity to inhibition by malonyl-CoA.

Thyroid-hormone deficiency in human subjects is associated with alterations in plasma lipoproteins that may contribute to increased risk of coronary vascular disease in this conditions (Starr, 1978). In addition to the well-documented hypercholesterolaemia, human hypothyroidism is generally, though not invariably, associated with elevated plasma triacylglycerol concentrations (O'Hara *et al.*, 1966; Nikkilä & Kekki, 1972; Tulloch *et al.*, 1973; Rossner & Rosenqvist, 1974; Mishkel & Crowther, 1976; Pykalistö *et al.*, 1976; Wahlqvist *et al.*, 1977). A substantial decrease in the rate of triacylglycerol clearance from the circulation almost certainly contributes to hypertriglyceridaemia in human hypothyroidism (Nikkilä & Kekki, 1972; Tulloch *et al.*, 1973; Pykalistö *et al.*, 1976). However, a more recent series of studies (Keyes & Heimberg, 1979; Keyes *et al.*, 1979, 1981) suggest that alterations in hepatic lipid metabolism may also contribute to this condition. In perfused livers from rats made hypothyroid with propylthiouracil there is enhanced synthesis and secretion of very-low-density-lipoprotein triacylglycerols and decreased formation of the products of fatty acid oxidation (CO_2 and ketone bodies) compared with pair-fed controls. Conversely, the production of ketone bodies is increased and the formation of triacylglycerols decreased in

the livers of rats treated with tri-iodothyronine. Thyroid hormone thus appears to direct hepatic fatty acid metabolism away from pathways of esterification and into oxidative pathways (Keyes & Heimberg, 1979; Keyes *et al.*, 1979, 1981). There have so far been few studies that could explain this redirection of fatty acid metabolism at the enzymic level. In rat liver the activities of enzymes associated with the pathway of triacylglycerol synthesis are not decreased on administration of thyroid hormones (Young & Lynen, 1969; Roncari & Murthy, 1975; Glenny & Brindley, 1978). On the other hand, both the hepatic carnitine content (Pande & Parvin, 1980) and CPT activity (Van Tol, 1975) are increased after administration of thyroxine to rats. Both of these changes could contribute to diversion of hepatic fatty acid metabolism towards oxidative processes. It was therefore decided to investigate the effect of the hypothyroid state on the activity and properties of liver CPT. The overt form of this enzyme (CPT_1) in rat liver mitochondria shows interesting regulatory properties (McGarry & Foster, 1980; Saggerson & Carpenter, 1981a, 1982), particularly with regard to its inhibition by malonyl-CoA. This effect is thought to contribute to the mechanism(s) whereby rates of hepatic fatty acid synthesis and oxidation are inversely related in a number of states (McGarry & Foster, 1980). It is possible that thyroid hormones might modify this mechanism since, paradoxically, both increased fatty acid synthesis and oxidation are observed in livers of

Abbreviations used: COT, carnitine acyltransferase activity with octanoyl-CoA; CPT, carnitine palmitoyltransferase (EC 2.3.1.21); CPT_1 , the overt form of carnitine palmitoyltransferase.

hyperthyroid animals (Spirtes *et al.*, 1953; Diamant *et al.*, 1972; Roncari & Murthy, 1975; Keyes *et al.*, 1981). In the euthyroid rat, starvation results in two changes in hepatic CPT. Firstly, the measured activity is increased (Norum, 1965; Aas & Daae, 1971; Harper & Saggerson, 1975; Van Tol, 1975; Bremer, 1981). This change is particularly seen in the overt activity (Harper & Saggerson, 1975; Bremer, 1981) and does not appear to involve synthesis of new protein (Norum, 1965). Secondly, the inhibitory effect of malonyl-CoA on hepatic CPT₁ is considerably decreased after starvation (Saggerson & Carpenter, 1981a,b; Bremer, 1981). Adrenal hormones do not appear to be involved in mediating this effect of starvation (Saggerson & Carpenter, 1981a), nor do pancreatic hormones appear to regulate sensitivity of the enzyme to malonyl-CoA (Saggerson & Carpenter, 1981a; Saggerson *et al.*, 1981). It was therefore also decided both to investigate the effect of thyroidectomy on the sensitivity of liver CPT₁ to malonyl-CoA and to determine whether the hypothyroid state modifies the changes in properties and activity normally seen in starvation.

Materials and methods

Animals

These were male Sprague-Dawley rats bred in the Animal Colony at University College London, and maintained on GR3-EK cube diet (E. Dixon and Sons, Ware, Herts., U.K.). Thyroidectomy was performed under chloral hydrate anaesthesia (0.4 g/kg body wt.) at 5 weeks of age (130–150 g body wt.). Animals were killed when aged 12 weeks. Only thyroidectomized animals showing a lower weight gain (approx. 80–90 g) over the intervening 7 weeks were used. Control animals grew by 160 g (approx.) in the same period. Starvation was commenced by removal of food at 10:00 h 1 day before they were killed. Drinking water for thyroidectomized rats was supplemented with calcium lactate (0.33 μ M).

Chemicals

Palmitoyl-CoA, octanoyl-CoA, malonyl-CoA and acetyl-CoA were from International Enzymes (Windsor, Berks., U.K.). L-Carnitine hydrochloride and bovine plasma albumin (fraction V) were from Sigma Chemical Co. (Kingston-upon-Thames, Surrey, U.K.). Before use the albumin was subjected to a defatting procedure (Chen, 1967), with minor modifications (Saggerson, 1972). Oxaloacetate was from Boehringer Corp. (Lewes, E. Sussex, U.K.). DL-[Me-³H]Carnitine hydrochloride was from The Radiochemical Centre (Amersham, Bucks., U.K.).

Experimental methods

Liver mitochondria were isolated as described by Saggerson (1982) and finally suspended in 0.3 M-

sucrose medium containing 10 mM-Tris/HCl buffer (pH 7.4) and 1 mM-EGTA (4 ml per g original wt. of liver). The protein contents of these preparations were determined by the method of Lowry *et al.* (1951), with bovine plasma albumin as standard.

CPT₁ assays were performed within 30 min of the isolation of mitochondria. Intact mitochondria (50 μ l) were preincubated at 25°C for 2 min in 1.0 ml containing 220 mM-sucrose, 40 mM-KCl, 10 mM-Tris/HCl (pH 7.4), 1 mM-EGTA, fatty acid-poor albumin (1.3 mg/ml) and the indicated concentrations of palmitoyl-CoA and malonyl-CoA. The reaction was initiated by addition of 20 μ l containing 0.4 μ mol of L-carnitine and 1 μ Ci of DL-[Me-³H]carnitine. As discussed elsewhere (Saggerson & Carpenter, 1982) it was found to be unnecessary to include respiratory inhibitors. After 4 min the reaction was terminated with 1 ml of ice-cold 1.2 M-HCl followed by 2 ml of water-saturated butanol. After mixing and brief centrifugation (1000 g_{av} for 5 min) the resulting butanol layer was washed with 5 ml of butanol-saturated water, and 1.0 ml of this, containing palmitoyl[³H]carnitine, taken for scintillation counting in 10 ml of toluene containing 2,5-bis-(5-t-butylbenzoxazol-2-yl)thiophen (4 g/litre). The amounts of mitochondrial protein (means $\pm$ S.E.M., as μ g/ml) in these assays were: normal fed, 238 $\pm$ 12; normal starved, 244 $\pm$ 27; thyroidectomized fed, 317 $\pm$ 26; thyroidectomized starved, 389 $\pm$ 8. After sonication for 2 min at 0–2°C, total CPT activity was measured as indicated above, except that only 25 μ l of the mitochondrial preparation was used. COT activity was measured similarly, except that octanoyl-CoA was substituted for palmitoyl-CoA. All of these assays were linear with time and mitochondrial protein concentration.

Citrate synthase (EC 4.1.3.7) was assayed spectrophotometrically at 25°C and 412 nm in 1.0 ml containing 220 mM-sucrose, 40 mM-KCl, 10 mM-Tris/HCl (pH 7.4), 1 mM-EGTA, fatty acid-poor albumin (1.3 mg/ml), 50 μ M-acetyl-CoA, 0.1 mM-5,5'-dithobis-(2-nitrobenzoic acid) and 10 μ l of sonicated mitochondria (Saggerson, 1982). The reaction was initiated by addition of 50 nmol of oxaloacetate.

Results and discussion

Four preparations of mitochondria from each of the four states under investigation were used in the following experiments. CPT₁ was assayed over the range of 10–100 μ M-palmitoyl-CoA in the absence of malonyl-CoA (Fig. 1) and also, in three states, in the presence of 10 μ M-malonyl-CoA. The inhibitory effect of 10 μ M-malonyl-CoA at each of the tested concentrations of palmitoyl-CoA is shown in Fig. 2

Fig. 1. Effect of palmitoyl-CoA concentration on CPT₁ activity

L-Carnitine was present throughout at 400 μM. Similar measurements (not shown) were also made in the presence of 10 μM-malonyl-CoA. The values are means ± S.E.M. for four experiments in each case. The bars indicate S.E.M. ○, Normal, fed; ●, normal, starved for 24 h; □, thyroidectomized, fed; ■, thyroidectomized, starved for 24 h.

Fig. 2. Influence of palmitoyl-CoA concentration on inhibition of CPT₁ by malonyl-CoA

The values are derived from the experiment shown in Fig. 1. Malonyl-CoA was used at 10 μM. The values are means ± S.E.M. for four experiments in each case. Symbols are as for Fig. 1.

Table 1. Enzyme activities in liver mitochondria

In all cases these are shown as means ± S.E.M. for four experiments and are expressed as nmol/min per mg of mitochondrial protein. The same values of CPT₁ are shown in Fig. 1. Total CPT, total COT and citrate synthase were measured after sonication of the mitochondria at 0–2°C for 2 min. *, **, *** and **** indicate $P < 0.05$, < 0.01 and < 0.001 respectively for effects of starvation (Student's t test). †, ††, ††† and †††† indicate $P < 0.05$, < 0.02 , < 0.01 and < 0.001 respectively for effects of thyroidectomy.

State	Body wt. (g)	Liver wt. (g)	Mitochondrial- protein (mg/100 g body wt.)	CPT ₁ activity			Total CPT activity			Citrate synthase activity	
				With 40 μM- palmitoyl-CoA	With 100 μM- palmitoyl-CoA	With 100 μM- palmitoyl-CoA	With 40 μM- palmitoyl-CoA	With 100 μM- palmitoyl-CoA	Total COT activity	Total COT activity	Citrate synthase activity
Normal, fed	306 ± 4	11.3 ± 0.5	70	4.0 ± 0.4	5.6 ± 0.6	9.7 ± 1.1	10.5 ± 0.5	9.7 ± 1.1	16.2 ± 0.7	80 ± 3	80 ± 3
Normal, starved	283 ± 4***	7.5 ± 0.1****	56	5.6 ± 0.3*	9.2 ± 0.7***	12.3 ± 1.0	11.2 ± 0.3	12.3 ± 1.0	20.0 ± 1.1*	76 ± 4	76 ± 4
Thyroidectomized, fed	214 ± 3††††	6.0 ± 0.2††††	71	1.7 ± 0.1†††	2.3 ± 0.2†††	7.3 ± 1.0	8.7 ± 0.3†	7.3 ± 1.0	13.2 ± 0.8†	61 ± 1††††	61 ± 1††††
Thyroidectomized, starved	197 ± 4††††	4.1 ± 0.2****††††	64	3.7 ± 0.4****†††	6.5 ± 0.4****††	10.6 ± 0.5*	10.7 ± 0.5**	10.6 ± 0.5*	16.5 ± 0.8*†	60 ± 1†††	60 ± 1†††

for the normal fed, thyroidectomized fed and thyroidectomized starved states. In addition (Fig. 3), the inhibitory effects of a range of malonyl-CoA concentrations were tested at one palmitoyl-CoA concentration (40 μM) for each of the four states. Table 1 shows a statistical analysis of the changes seen in Fig. 1; two representative palmitoyl-CoA concentrations (40 and 100 μM) are used for this purpose. Table 2 shows values derived from Hill plots of the data from the individual 16 experiments. The Hill coefficient and $s_{0.5}$ (the concentration of palmitoyl-CoA giving half-maximal CPT₁ activity under the assay conditions) were obtained by regression analysis of the individual Hill plots, which in all cases were good-fit straight lines ($0.93 < r \leq 0.99$).

After thyroidectomy there was a very substantial decrease in the CPT₁ activity measured in mitochondria from fed animals (approx. 58% decrease at 40 μM - and 100 μM -palmitoyl-CoA; see Fig. 1 and Table 1). On starvation of euthyroid animals, CPT₁ activity was increased by 40% and 64% at 40 μM - and 100 μM -palmitoyl-CoA respectively. Starvation of thyroidectomized animals, however, resulted in 3-fold greater percentage increases in CPT₁ activity (118% and 183% increases respectively at 40 μM - and 100 μM -palmitoyl-CoA). In absolute terms the increase in CPT₁ activity on starvation of thyroidectomized animals was comparable with or slightly greater than that seen when euthyroid animals were starved. These activities were expressed relative to mg of mitochondrial protein. It may also be calculated from Table 1 that in the fed state thyroidectomy also decreased CPT₁ relative to the mitochondrial marker citrate synthase, even though thyroidectomy decreased citrate synthase activity by approx. 25%. The mitochondrial preparations were also sonicated and then re-assayed for CPT activity to obtain a measurement representative of the total (latent + overt) activity. This treatment obviously results in some alterations in kinetic properties, since total CPT activity was either unchanged or even

slightly decreased when the palmitoyl-CoA concentration was increased from 40 to 100 μM . This is clearly not the case with CPT₁ measured with whole mitochondria (Fig. 1 and Table 1). As found previously (Harper & Saggerson, 1975; Bremer, 1981), the change in CPT₁ activity on starvation was more pronounced than the change in the total activity. The same tendency was seen in the mitochondria from thyroidectomized animals (Table 1).

Mitochondria from liver and other tissues will readily form octanoylcarnitine from octanoyl-CoA + carnitine (Kopec & Fritz, 1971; Solberg, 1971, 1972; Saggerson & Carpenter, 1981b; Saggerson, 1982). It has been suggested that this capability

Fig. 3. Effect of malonyl-CoA concentration on CPT₁ activity

L-Carnitine was present throughout at 400 μM and palmitoyl-CoA at 40 μM . The absolute CPT₁ activities in the absence of malonyl-CoA are shown in Fig. 1 and Table 1. The values are means $\pm$ S.E.M. for four experiments in each case. Symbols are as for Fig. 1.

Table 2. Effects of starvation, thyroidectomy and malonyl-CoA on kinetic properties of CPT₁ in liver mitochondria. The values are calculated from Hill plots of the data from the individual experiments summarized in Figs. 1 and 2. These are expressed as means $\pm$ S.E.M. for four experiments. *, ** and *** indicate $P < 0.05$, < 0.02 and < 0.01 respectively for effects of starvation. † and †† indicate $P < 0.05$ and < 0.01 respectively for effects of thyroidectomy. ‡ and ‡‡ indicate $P < 0.01$ and < 0.001 respectively for effects of malonyl-CoA.

Concn. of malonyl-CoA (μM)		Normal, fed	Normal, starved	Thyroidectomized, fed	Thyroidectomized, starved
0	Hill coefficient	2.47 ± 0.11	2.47 ± 0.16	$1.74 \pm 0.14^{\dagger\dagger}$	$2.65 \pm 0.10^{***}$
	$s_{0.5}$ for palmitoyl-CoA	$26.6 \pm 0.3 \mu\text{M}$	$30.7 \pm 1.3 \mu\text{M}^*$	$21.2 \pm 2.0 \mu\text{M}^{\dagger}$	$28.2 \pm 0.7 \mu\text{M}^{**}$
10	Hill coefficient	2.62 ± 0.18	—	2.12 ± 0.11	$2.67 \pm 0.09^{***}$
	$s_{0.5}$ for palmitoyl-CoA	$42.5 \pm 3.4 \mu\text{M}^{\dagger}$	—	$41.6 \pm 1.8 \mu\text{M}^{\dagger\dagger}$	$33.6 \pm 1.1 \mu\text{M}^{***\dagger}$

(COT activity) resides in an enzyme entity that is separate from carnitine acetyltransferase and CPT (Kopeck & Fritz, 1971; Solberg, 1971, 1972), although this interpretation has been disputed subsequently (Clarke & Bieber, 1981). Nevertheless, rat liver COT activity shows a number of marked differences in properties from CPT (Saggerson & Carpenter, 1981a, 1982; Saggerson, 1982). Table 1 shows that starvation increased total COT activity measured in sonicated preparations of mitochondria from normal and thyroidectomized animals and that thyroidectomy decreased this activity in both the fed and starved states.

As found previously (Saggerson & Carpenter, 1981c, 1982), with the assay conditions employed, the relationship between CPT₁ activity and palmitoyl-CoA concentration was sigmoidal rather than hyperbolic (Fig. 1). As also seen previously (Saggerson & Carpenter, 1981c), inhibition by malonyl-CoA was seen at lower concentrations of palmitoyl-CoA, but not at the highest tested concentrations of this substrate (Fig. 2). Table 2 shows that, in the fed state, thyroidectomy resulted in appreciable changes in the kinetic properties of CPT₁, in that, in the absence of malonyl-CoA, both the Hill coefficient and the $s_{0.5}$ for palmitoyl-CoA were decreased. Starvation increased the $s_{0.5}$ for palmitoyl-CoA in the normal and thyroidectomized states and also substantially increased the Hill coefficient in the thyroidectomized state. The reason for the changes in kinetics after thyroidectomy (fed state) are unknown, but it is noteworthy that qualitatively similar changes in CPT properties are seen after treatment of mitochondria with detergent or with thiol-group reagents (Saggerson, 1982; Saggerson & Carpenter, 1982). Malonyl-CoA (10 μ M) considerably increased the $s_{0.5}$ for palmitoyl-CoA in both fed states. Although some increase in this parameter was seen with malonyl-CoA in the starved thyroidectomized state, the change was far less than in the fed state and is indicative of the smaller effect of this inhibitor in the starved state (Saggerson & Carpenter, 1981c). A similar diminution in the effect of malonyl-CoA on the $s_{0.5}$ for palmitoyl-CoA after starvation in the euthyroid state may be deduced from the data shown by Saggerson & Carpenter (1981c).

Fig. 2 restates the finding that starvation of thyroidectomized rats results in a diminished inhibitory effect of malonyl-CoA. This is seen over a wide range of palmitoyl-CoA concentrations. Fig. 2 also implies that, in the fed state, there is no gross difference between the hypothyroid and euthyroid states in the response of CPT₁ to malonyl-CoA. The same may be deduced from Fig. 3, which again demonstrates that normal thyroid status is not necessary for the decrease in sensitivity to malonyl-CoA seen in starvation. The data of Fig. 3, taken in

conjunction with those of Table 1, are also noteworthy in another respect. McGarry *et al.* (1978) have suggested that the latent form of the enzyme (CPT₂) is relatively insensitive to malonyl-CoA. Bremer (1981) has speculated that starvation leads to a decrease in the latency of malonyl-CoA-insensitive CPT, thus accounting for the increase in overt CPT and the decrease in malonyl-CoA-sensitivity. This is unlikely in hypothyroidism, since starvation in the hypothyroid state results in a far greater percentage change in CPT₁ than in the euthyroid state, whereas the change in malonyl-CoA sensitivity on starvation is broadly similar in both thyroid states. It is also noteworthy that, although the absolute activities of CPT₁ are very different in the fed euthyroid and hypothyroid states, the sensitivities to malonyl-CoA are again similar. These findings strongly suggest that the extent of CPT latency and the malonyl-CoA-sensitivity are not directly linked properties.

There are two main conclusions from this study. Firstly, normal thyroid status does not appear to be necessary for either of the previously noted changes in hepatic CPT seen in starvation (increased CPT₁ activity with decreased malonyl-CoA-sensitivity). Secondly, the hypothyroid state appears to result in a substantial decrease in CPT₁ activity both per mg of mitochondrial protein and, since the total mitochondrial protein is only slightly changed (Table 1), per 100 g body wt. It is suggested that this may contribute to the diversion of hepatic fatty acid metabolism away from oxidation towards esterification pathways seen in hypothyroidism.

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