

CARNITINE PALMITOYLTRANSFERASE AND CARNITINE OCTANOYLTRANSFERASE ACTIVITIES IN LIVER, KIDNEY CORTEX, ADIPOCYTE, LACTATING MAMMARY GLAND, SKELETAL MUSCLE AND HEART

Relative activities, latency and effect of malonyl CoA

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1. Introduction

Transfer of long chain fatty acids through the inner membrane of mitochondria appears to require two pools of carnitine palmitoyl transferase (CPT), one latent, the other overt [1–5]. The relative proportions of these latent and overt forms of CTP has mainly been studied in liver mitochondria. A medium chain carnitine acyltransferase (COT, carnitine octanoyltransferase) has also been reported in heart and liver [6,7]. This activity appears to be distinct from CPT and from carnitine acetyltransferase (CAT), but its physiological role is uncertain. Until recently [8], it had not been established whether COT in heart or liver mitochondria showed latency.

There has been considerable interest in the observation that overt CPT activity in liver mitochondria is potentially inhibited by malonyl CoA [9]. CPT activity in heart mitochondria [8] and COT in liver [10] and heart [8] mitochondria are also extremely sensitive to malonyl CoA. It was therefore of interest to investigate the sensitivity of these activities to malonyl CoA in a wide range of tissues to see if regulation by this effector has general applicability. The relative activities of the latent and overt forms of CPT and COT have also been measured and are found to vary widely from tissue to tissue.

2. Materials and methods

Coenzyme A thioesters were from PL-Biochemicals, oxaloacetate from Boehringer and L-carnitine from Sigma. Tissues were obtained from male

Sprague-Dawley rats (160–180 g) except for mammary gland which was from female Sprague-Dawley rats in peak lactation (12 days) suckling their first litter. Single livers, kidney cortices from 2 rats, hearts from 4 rats or mammary gland from a single rat were homogenised in ice-cold 0.25 M sucrose medium containing 10 mM Tris–HCl (pH 7.4) defatted albumin (10 mg/ml) and 1 mM EGTA using a Potter-Elvehjem homogeniser fitted with a motor-driven teflon pestle (radical clearance, 0.2 mm). Before homogenisation, mammary gland was forced through a tissue press. Muscle, containing as far as possible red fibres, was collected from the rear legs of 3 rats and homogenised as above except that 0.15 M KCl containing 10 mM Tris–HCl (pH 7.4) defatted albumin (10 mg/ml) and 1 mM EGTA was substituted for the sucrose–Tris homogenisation buffer. Adipocytes were isolated by disaggregation of the epididymal fat pads of ten rats using collagenase [11] and were broken by swirling in a glass tube on a vortex mixer [12] with 30 ml ice-cold 0.25 M sucrose 10 mM Tris–HCl (pH 7.4) defatted albumin (10 mg/ml), 1 mM EGTA. Mitochondria from all tissues were isolated and washed as in [10]. Washing of all types of mitochondria was with 0.25 M sucrose, 10 mM Tris–HCl (pH 7.4), 1 mM EGTA. Finally, the mitochondria were resuspended in 0.3 M sucrose, 10 mM Tris–HCl (pH 7.4), 1 mM EGTA. The protein content of these stock mitochondrial suspensions was determined [13] using bovine serum albumin as standard and was found to be (means values as mg/ml): liver, 4.0; kidney cortex, 3.4; adipocyte, 2.0; mammary gland, 3.3; skeletal muscle, 4.0; heart, 2.7.

For assay of CPT (EC 2.3.1.21), 50 μ l fresh whole mitochondria or of mitochondria subjected to sonication for 2 min were preincubated at 25°C in 1.0 ml containing 220 mM sucrose, 40 mM KCl, 10 mM Tris-HCl (pH 7.4), 1 mM EGTA, defatted albumin (1.3 mg/ml), 0.5 mM dithiothreitol, 40 μ M palmitoyl CoA and the required concentration of malonyl CoA. The reaction was initiated by addition of 20 μ l containing 0.4 μ mol L-carnitine and 1 μ Ci D,L[methyl- 3 H] carnitine. After 4 min the reaction was terminated with 1 ml ice-cold 1.2 M HCl followed by 2 ml of water-saturated butanol. After mixing and brief centrifugation, the butanol layer was washed with 5 ml butanol-saturated water and 0.1 ml of the butanol layer, containing acyl [3 H]carnitine taken for liquid scintillation during counting. Zero time blanks were measured and subtracted.

COT was assayed as for CPT except that 40 μ M CoA was substituted for palmitoyl CoA.

Carnitine acetyltransferase (CAT, EC 2.3.1.7) and citrate synthase (EC 4.1.3.7) were assayed spectrophotometrically at 412 nm and 25°C in whole and sonicated mitochondria in 1.0 ml containing 220 mM sucrose, 40 mM KCl, 10 mM Tris-HCl (pH 7.4), 1 mM EGTA, defatted albumin (1.3 mg/ml), 50 μ M acetyl CoA and 0.1 mM 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB). CAT assays were initiated by addition of 400 nmol L-carnitine and citrate synthase by addition of 60 nmol oxaloacetate.

3. Results and discussion

Fig.1 shows that overt CPT activities in all the tested tissues were inhibited by malonyl CoA. However, there was a clear distinction between heart and skeletal muscle on one hand and liver, kidney cortex and mammary gland on the other in that malonyl CoA was effective at concentrations 1–2 orders of magnitude lower in the former types of mitochondria. For these 5 types of mitochondria, plots of (percentage inhibition by malonyl CoA) $^{-1}$ vs (malonyl CoA concentration) $^{-1}$ were linear, suggesting a hyperbolic-type relationship between the concentration of malonyl CoA and the effect of this inhibitor. Overt CPT in adipocyte mitochondria was anomalous in that the above relationship was not obeyed over the whole of the tested range of malonyl CoA concentrations. It may be seen in fig.1 that the response of adipocyte CPT to malonyl CoA appears to be a com-

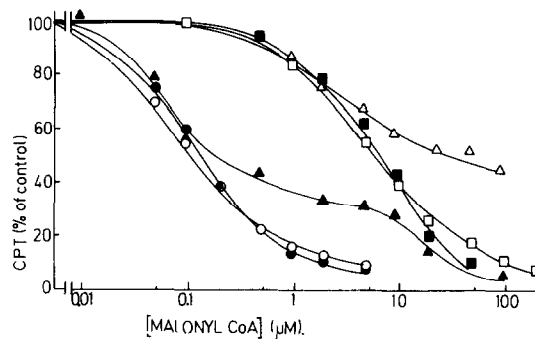


Fig.1. Effect of malonyl CoA on overt CPT activity. CPT assays were performed with whole mitochondria. The values are expressed as percentage of the activities shown in table 1 and are the means of the number of experiments shown in table 1. (□) Liver; (■) kidney cortex; (▲) adipocyte; (△) mammary gland; (●) skeletal muscle; (○) heart.

bination of a highly sensitive component (muscle-like) and a less sensitive component (liver or kidney-like). It is tempting to speculate that heart and skeletal muscle mitochondria might contain an isoenzyme of CPT differing from that found in the liver or kidney cortex. If this were so, the adipocyte activity might be a composite of these. It is noteworthy that liver and heart CPT differ from each other in a number of other properties [8].

We are unaware of any measurement of malonyl CoA levels in tissues other than liver at the present time. It is reasonable to expect this metabolite to be present in lipogenic tissues such as adipocytes or lactating mammary gland. A role for malonyl CoA in heart, kidney or skeletal muscle is less certain. However, any condition, whether normal or pathological in which malonyl CoA was formed in these tissues should be associated with inhibition of CPT. Such an effect could be particularly profound in the heart or in skeletal muscle.

Fig.2 shows that overt COT activity in skeletal muscle and heart mitochondria was inhibited by malonyl CoA concentrations similar to those that inhibit CPT in these tissues. However, only ~50% of this COT activity appeared to be inhibitable. Kidney COT was more sensitive to malonyl CoA than kidney CPT by ~1 order of magnitude. As shown in [10], liver COT was inhibited by malonyl CoA, but there appeared to be two components to this inhibition curve. Mammary gland COT was barely affected by malonyl CoA.

The data shown in fig.1,2 were normalised values

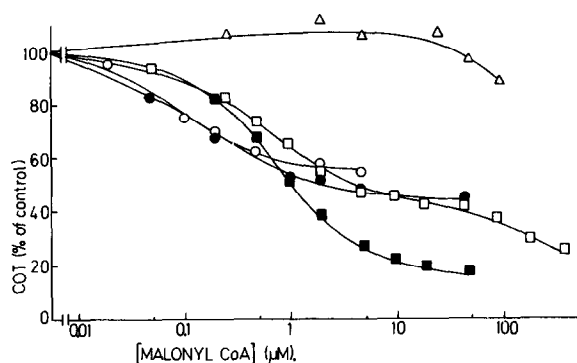


Fig.2. Effect of malonyl CoA on overt COT activity. COT activities were performed with whole mitochondria. The values are expressed as percentages of the activities shown in table 1 and are the means of the number of experiments shown in table 1. Symbols are as for fig.1.

obtained by expression of the activities in the absence of malonyl CoA as 100%. Table 1 shows that the absolute values of these activities varied considerably between the different mitochondrial types. Heart and skeletal muscle overt CPT activities were the highest and represented a large proportion of the total that was measurable after sonication. Liver and kidney cortex CPTs were intermediate in activity. Liver

and kidney differed however in that a quite large proportion of the liver activity was latent whereas in the kidney most of the activity appeared to be overt. Adipocyte and mammary gland overt CPT activities were low and a very large proportion of the total CPT in these tissues was latent. There was no general parallelism between the overt or latent activities of COT compared with CPT between the various tissues, nor was there any parallelism between CPT and COT in the extent to which the ratio of overt/latent activities varied from tissue to tissue. It is particularly noteworthy that in kidney all the COT appeared to be overt and that in skeletal muscle or heart latent COT exceeded the overt activity whereas the opposite was found for CPT in these tissues.

Citrate synthase and carnitine acetyl transferase activities were measured in an assay medium similar in composition to that used for CPT and COT. In general these activities were increased ≥ 10 -fold by sonication suggesting that reasonable intactness of the mitochondria was retained. Palmitoyl CoA at $40 \mu\text{M}$, which was used in the assay of CPT, did not appear to diminish the latency of citrate synthase. The possibility that this concentration of palmitoyl CoA might render the mitochondria 'leaky' and thereby be the cause of the smallness of the latent proportion of

Table 1
Activities of CPT, COT, CAT and citrate synthase in mitochondria

		CPT	COT	CAT	Citrate synthase	
						with $40 \mu\text{M}$ palmitoyl CoA
Overt						
Liver	[4]	2.90 ± 0.35	3.65 ± 0.22	—	2.79 ± 0.42	2.53 ± 0.17
Kidney	[4]	2.75 ± 0.22	3.71 ± 0.05	2.80 ± 0.31	13.0 ± 1.8	13.9 ± 2.3
Adipocyte	[5]	0.64 ± 0.07	1.30 ± 0.35	—	—	—
Mammary gland	[4]	0.34 ± 0.08	0.39 ± 0.06	0	55.6 ± 6.9	52.6 ± 4.5
Skeletal muscle	[4]	6.73 ± 0.31	2.72 ± 0.26	7.25 ± 1.26	47.1 ± 6.0	45.7 ± 5.1
Heart	[4]	9.33 ± 0.37	3.77 ± 0.11	7.80 ± 2.21	55.0 ± 2.0	51.1 ± 1.2
Total						
Liver	[4]	6.84 ± 0.63	5.98 ± 0.31	—	34.7 ± 1.1	33.6 ± 1.5
Kidney	[4]	3.45 ± 0.34	3.59 ± 0.44	20.51 ± 2.24	118 ± 4	115 ± 3
Adipocyte	[5]	9.46 ± 1.52	6.38 ± 1.34	—	—	—
Mammary gland	[4]	7.34 ± 0.14	3.16 ± 0.55	4.30 ± 1.28	688 ± 88	659 ± 93
Skeletal muscle	[4]	8.07 ± 0.31	6.52 ± 0.41	82.3 ± 2.9	520 ± 21	502 ± 17
Heart	[4]	16.18 ± 0.15	8.63 ± 0.11	72.0 ± 11.1	649 ± 16	578 ± 4

Overt activities were measured with freshly prepared whole mitochondria. Total activities were measured after sonication of mitochondria for 2 min. The values are means \pm SEM of the no. expt in brackets and are expressed as $\text{nmol} \cdot \text{min}^{-1} \cdot \text{mg mitochondrial protein}^{-1}$

the CPT in kidney, heart or skeletal muscle can therefore be discounted.

The proportion of CPT that is latent in this study in liver, adipocyte or heart mitochondria is somewhat larger than found in other studies [8,14] in which a DTNB-linked assay was used. Also the sensitivity of heart CPT to malonyl CoA reported here is greater than found in [8] with a DTNB-linked assay. DTNB appears to diminish the sensitivity of CPT to malonyl CoA and to increase the proportion of the total CPT that is overt (E. D. S., unpublished).

This study demonstrates considerable differences between different mitochondrial types with regard to the intramitochondrial distribution of CPT and COT and the sensitivity of these activities to malonyl CoA. Presumably these findings are of physiological significance, but at present this is unclear.

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