Sensitivity of brown-adipose-tissue carnitine palmitoyltransferase to inhibition by malonyl-CoA

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Overt carnitine palmitoyltransferase in mitochondria isolated from interscapular brown adipose tissue of cold-adapted rats or rats maintained at normal temperature is extremely sensitive to inhibition by malonyl-CoA.

CPT₁ in rat liver mitochondria is inhibited by low concentrations of malonyl-CoA (McGarry *et al.*, 1978). It is suggested that this effect offer a mechanism by which rates of hepatic fatty acid oxidation (and hence ketogenesis) may be inversely related to rates of fatty acid synthesis (McGarry & Foster, 1980). More recently it was shown that CPT₁ activity in mitochondria from various other rat tissues can also be inhibited by low concentrations of malonyl-CoA (Saggerson & Carpenter, 1981*b*). This effect was seen with CPT₁ from lipogenic tissues such as white adipocytes and lactating mammary gland and also in oxidative tissues, e.g. heart and skeletal muscle. Surprisingly, CPT₁ from these muscle tissues was approx. 10–15 times more sensitive to malonyl-CoA than was CPT₁ from liver. The malonyl-CoA content of extrahepatic tissues is unknown at present, and may be extremely difficult to quantify.

Thermogenesis in brown adipose tissue is accompanied by a high rate of fatty acid oxidation; for review see Nicholls (1979). Measurements *in vivo* in the cold-adapted rat or in animals maintained at normal temperatures suggest that brown adipose tissue may also be extremely active in fatty acid synthesis (McCormack & Denton, 1977; Trayhurn, 1979; Agius & Williamson, 1980). It was therefore decided to establish whether brown-adipose-tissue CPT₁ was sensitive to inhibition by malonyl-CoA. It was expected that this control mechanism might not be operative, at least in the cold-adapted state. Surprisingly, the opposite was found; the enzyme in this tissue is extremely sensitive to this metabolite.

Abbreviations used: CPT₁, the overt form of carnitine palmitoyltransferase (EC 2.3.1.21); CPT₂, the latent form of carnitine palmitoyltransferase; CPT, carnitine palmitoyltransferase.

Materials and methods

Source of chemicals are described by Saggerson (1982). Brown adipose tissue was obtained from male Sprague–Dawley rats. Normal animals were maintained at 21°C for 6 weeks from birth and weighed 170–190 g at the time of experiment. Cold-adapted animals were maintained at 21°C for 25 days after birth (until 4 days after weaning) and then kept for a further 21 days at 4–5°C. These animals were killed in the cold. At the time of experiment these had an average body weight of 203 g. All animals had constant access to cube diet and drinking water and were maintained on a light/dark cycle with light from 06:00 to 19:00 h (13 h). The interscapular brown adipose tissues from six to eight normal or from two or three cold-adapted rats were trimmed free as far as possible of adhering muscle or white fat, and the pooled tissues homogenized with a Potter–Elvehjem homogenizer fitted with a motor-drive Teflon pestle (radial clearance 0.2 mm). Homogenization was in ice-cold 0.25 M-sucrose containing 10 mM-Tris/HCl (pH 7.4), 1 mM-EGTA and fatty acid-poor albumin (10 mg/ml). Mitochondria were isolated and washed as described by Saggerson & Carpenter (1981*a*). Finally, the mitochondria were resuspended in 0.3 M-sucrose/10 mM-Tris/HCl (pH 7.4)/1 mM-EGTA. The protein contents of these stock mitochondrial suspensions were determined by the method of Lowry *et al.* (1951), with bovine serum albumin as a standard, and were found to be (mean values as mg/ml): from normal rats, 3.3; from cold-adapted rats 4.2. Samples (50 µl) of fresh (within 30 min of isolation) whole mitochondria or of mitochondria sonicated for 2 min were assayed for CPT activity by following the incorporation of [³H]carnitine into palmitoyl[³H]carnitine. This was performed at 25°C in a 1.0 ml reaction mixture

(Saggerson & Carpenter, 1981a; Saggerson, 1982). The assays were linear with time and amounts of mitochondria. Zero-time blanks were measured and subtracted. Citrate synthase (EC 4.1.3.7) was assayed as described by Saggerson & Carpenter (1981a).

Results and discussion

In other studies (Saggerson & Carpenter, 1981c; Saggerson, 1982) it has been demonstrated that, under the assay conditions used here, the relationship between heart or liver CPT₁ activity and [palmitoyl-CoA] is sigmoidal. Malonyl-CoA increases this apparent sigmoidicity, but does not appear to alter the maximum velocity. In the absence of malonyl-CoA, the kinetics with palmitoyl-CoA are more sigmoidal with heart CPT₁ than with the liver enzyme (Saggerson & Carpenter, 1982). Fig. 1 shows that, like heart CPT₁, brown-adipose-tissue CPT₁ activity showed a pronounced sigmoidal dependence on [palmitoyl-CoA] even in the absence of malonyl-CoA. Low concentrations of malonyl-CoA considerably inhibited the activity at low [palmitoyl-CoA] and increased the apparent sigmoidicity. This experiment was performed with mitochondria from rats maintained at normal temperature. Similar sigmoidicity in this curve was seen with CPT₁ in mitochondria from cold-adapted rats. This treatment slightly decreased the CPT₁ activity per mg of protein (see also the legend to Fig. 2), but did not alter the concentration of palmitoyl-CoA giving half-maximal activity (38 μ M) under these assay conditions. In the absence of malonyl-CoA Hill coefficients were 3.2 and 3.0 in the normal and cold-adapted states respectively. The activity per mg of protein of brown-adipose-tissue CPT in the absence of malonyl-CoA was comparable with that found under these assay conditions in other oxidative tissues, such as heart or skeletal muscle (Saggerson & Carpenter, 1981b, 1982) and higher than that found in liver, kidney, white adipocytes or mammary gland (Saggerson & Carpenter, 1981b). Reasonable mitochondrial intactness was suggested by the fact that CPT₁ activity was increased by 75% on sonication (see the legend to Fig. 2) and by the observation that measurable citrate synthase activity increased 9.4-fold, from 142 to 1342 nmol/min per mg of protein, on sonication. This citrate synthase activity is incidentally twice that found in mitochondria from rat mammary gland, skeletal muscle or heart (Saggerson & Carpenter, 1981b) and is matched only by the very high activity found in mitochondria from white adipocytes (Harper & Saggerson, 1975) or brain (Land *et al.*, 1977).

Fig. 2 compares the malonyl-CoA sensitivity of brown-adipose-tissue CPT₁, assayed with 40 μ M-palmitoyl-CoA, in the normal and cold-adapted

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

L-Carnitine was present throughout at 400 μ M. Results are for mitochondria from normal rats (21°C) with [malonyl-CoA] at: ●, zero; △, 0.5 μ M; ○, 5 μ M, and mitochondria from cold-adapted rats (4°C) with [malonyl-CoA] at zero (■). Values are means of three experiments in each case; the bars indicate $\pm$ S.E.M. Where not shown, these lie within the symbol.

states. Surprisingly, cold-adaptation for 3 weeks resulted in no change in this effect. The sensitivity of brown-adipose-tissue CPT₁ to malonyl-CoA was similar to that found with the enzyme from heart or skeletal muscle, but was approx. 50 times the sensitivity of the liver enzyme (see Fig. 1 of Saggerson & Carpenter, 1981b). In both conditions approx. 20% of the measured brown-adipose-tissue CPT was insensitive to malonyl-CoA even at the highest concentrations tested. It is possible that a proportion of the mitochondria were damaged, thereby allowing access of the assay substrates to the CPT₂ activity, which, in liver mitochondria, is reported to be insensitive to malonyl-CoA (McGarry *et al.*, 1978). However, the 20% proportion of the CPT activity that was malonyl-CoA-insensitive was most reproducibly and consistently observed in each mitochondrial preparation. It is unlikely that such a constant proportion of the mitochondria could be damaged from preparation to preparation.

As indicated above, interscapular brown adipose tissue from cold-adapted rats appears to have a high

Fig. 2. Effect of malonyl-CoA concentration on CPT₁ activity

L-Carnitine and palmitoyl-CoA were present throughout at 400 μM and 40 μM respectively. O, Normal rats (21°C); ●, cold-adapted rats (4°C). The values are means of three experiments and are expressed as percentages of the activity in the absence of malonyl-CoA which were 8.2 ± 0.3 and 6.7 ± 0.2 nmol/min per mg of protein for the normal and cold-adapted states respectively. The bars indicate $\pm$ S.E.M. Where not shown, these lie within the symbol. All tested concentrations of malonyl-CoA significantly ($P < 0.05$) inhibited CPT₁. Sonication for 2 min yielded total (CPT₁ + CPT₂) activities of 14.4 ± 1.0 and 11.8 ± 0.2 nmol/min per mg of protein respectively for the normal and cold-adapted states.

rate of lipogenesis *in vivo* as measured by the incorporation of ³H from injected ³H₂O into fatty acids in the tissue. It is of course possible that a proportion of this labelled fatty acid was derived from lipogenesis in other tissues, e.g. liver. However, the contention that brown adipose tissue is potentially active in lipogenesis is supported by the finding that the tissue has extremely high activities of pyruvate dehydrogenase and acetyl-CoA carboxylase (McCormack & Denton, 1977). Clearly there is a conundrum here. Malonyl-CoA must be present in

brown-fat cells in order to sustain the observed high rates of lipogenesis. How can this be compatible with a simultaneous high rate of β-oxidation?

Measurements of the tissue content of malonyl-CoA will be necessary to establish the extent to which CPT₁ might be inhibited in brown adipose tissue and to establish whether the content of this metabolite changes in different states. The presence of malonyl-CoA in the tissue, even if invariant, could bring about a highly sigmoidal relationship between CPT₁ activity and the content of acyl-CoA substrates, hence rendering the rate of acyl-group entry into β-oxidation highly sensitive to small changes in the amounts of these substrates. Alternatively, or additionally, other metabolites, as yet unrecognized, may also play a role in the regulation of CPT₁, perhaps to oppose the effect of malonyl-CoA.

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