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Interacting effects of L-carnitine and malonyl-CoA on rat liver carnitine palmitoyltransferase

Michael I. BIRD and E. David SAGGERSON*
**Department of Biochemistry, University College London, Gower Street, London WC1E 6BT, U.K.*

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1. Malonyl-CoA significantly increased the K_m for L-carnitine of overt carnitine palmitoyltransferase in liver mitochondria from fed rats. This effect was observed when the molar palmitoyl-CoA/albumin concentration ratio was low (0.125–1.0), but not when it was higher (2.0). In the absence of malonyl-CoA, the K_m for L-carnitine increased with increasing palmitoyl-CoA/albumin ratios. 2. Malonyl-CoA did not increase the K_m for L-carnitine in liver mitochondria from 24h-starved rats or in heart mitochondria from fed animals. 3. The K_m for L-carnitine of the latent form of carnitine palmitoyltransferase was 3–4 times that for the overt form of the enzyme. 4. At low ratios of palmitoyl-CoA/albumin (0.5), the concentration of malonyl-CoA causing a 50% inhibition of overt carnitine palmitoyltransferase activity was decreased by 30% when assays with liver mitochondria from fed rats were performed at 100μM-instead of 400μM-carnitine. Such a decrease was not observed with liver mitochondria from starved animals. 5. L-Carnitine displaced [^{14}C]malonyl-CoA from liver mitochondrial binding sites. D-Carnitine was without effect. L-Carnitine did not displace [^{14}C]malonyl-CoA from heart mitochondria. 6. It is concluded that, under appropriate conditions, malonyl-CoA may decrease the effectiveness of L-carnitine as a substrate for the enzyme and that L-carnitine may decrease the effectiveness of malonyl-CoA to regulate the enzyme.

The overt form of carnitine palmitoyltransferase (CPT_1) in a number of mammalian tissues shows interesting regulatory properties in that it is strongly inhibited by malonyl-CoA (McGarry *et al.*, 1978; Saggerson & Carpenter, 1981*b*, 1982*a*) and, less potently, by several other short-chain CoA thioesters (Mills *et al.*, 1983). The inhibitory effect of malonyl-CoA in liver mitochondria is less pronounced after starvation (Bremer, 1981; Saggerson & Carpenter, 1981*a*,*b*; Robinson & Zammit, 1982; Saggerson *et al.*, 1982). Several studies have shown that the inhibitory effect of malonyl-CoA is interactive with one of the substrates of the CPT_1 reaction, namely palmitoyl-CoA (McGarry *et al.*, 1978; Bremer, 1981). Malonyl-CoA increases the $s_{0.5}$ for palmitoyl-CoA without changing the $V_{max.}$ (Saggerson & Carpenter, 1981*c*, 1982*b*; Saggerson, 1982; Saggerson *et al.*, 1982). Further evidence for interaction between malonyl-CoA and the acyl-CoA substrate comes from studies of [^{14}C]malonyl-CoA binding to isolated mitochondria. Palmitoyl-CoA displaces [^{14}C]malonyl-CoA from high-affinity binding sites on mitochondria from rat skeletal muscle (Mills *et al.*, 1983, 1984), heart and liver (Bird & Saggerson, 1984).

The hepatic content of carnitine, the other substrate of CPT_1 in the 'physiological' direction, is increased both in short-term ketotic states (3h after glucagon or anti-insulin serum administration) and in long-term states such as starvation or alloxan-diabetes (McGarry *et al.*, 1975). Although it has been speculated that the binding sites on CPT_1 for carnitine and malonyl-CoA might be closely associated (Mills *et al.*, 1984), it has also been proposed (McGarry *et al.*, 1977; Veerkamp & Van Moerkerk, 1982) that the inhibitory effect of

Abbreviations used: CPT_1 and CPT_2, the overt and latent forms respectively of carnitine palmitoyltransferase (EC 2.3.1.21); $I_{max.}$, the maximum inhibition of CPT_1 activity by malonyl-CoA; IC_{50}, the concentration of malonyl-CoA causing 50% of $I_{max.}$; I_{50}, the concentration of displacing ligand causing 50% of maximal displacement of bound malonyl-CoA; $K_{D(1)}$, $K_{D(2)}$, N_1 and N_2, the dissociation constants and maximal binding capacities of high-affinity (1) and low-affinity (2) malonyl-CoA-binding sites.

* To whom reprint requests should be addressed.

malonyl-CoA is not altered by changing the concentration of carnitine. Here we report that L-carnitine and malonyl-CoA do interact in the hepatic CPT_1 system, provided that the assay conditions are appropriate. It is shown that malonyl-CoA increases the K_m of the enzyme for carnitine, and hence the inhibitory effect of malonyl-CoA varies with carnitine concentration. This effect is not apparent in the starved state in liver and is not seen in heart mitochondria. A further phenomenon reported here is the observation that low concentrations of L-carnitine can displace [^{14}C]malonyl-CoA from liver mitochondrial binding sites. It is not known to what extent this relates to the way carnitine and malonyl-CoA interact at the level of CPT_1 kinetics.

Materials and methods

Animals

These were male Sprague–Dawley rats bred at University College London and fed on GR3-EK cube diet (E. Dixon and Sons, Ware, Herts, U.K.). Starved animals received only water for 24h preceding the experiments, and all animals were killed between 09:30 and 11:00h.

Chemicals

Sources and treatment of chemicals were as described previously (Saggerson *et al.*, 1982; Bird & Saggerson, 1984).

Isolation of mitochondria

Liver and heart mitochondria were obtained as described by Saggerson (1982) and finally suspended in 0.3M-sucrose medium containing 10mM-Tris/HCl buffer (pH 7.4), 1mM-EGTA and 1mM-dithiothreitol to give protein concentrations of approx. 6 and 3mg/ml for liver and heart mitochondria respectively. Protein was measured by the method of Lowry *et al.* (1951), with bovine serum albumin as a standard.

Analytical methods

CPT_1 assays were performed within 30min of the isolation of mitochondria. Intact mitochondria (50μl) were preincubated at 25°C for 4min in 1.0ml containing 150mM-sucrose, 60mM-KCl, 25mM-Tris/HCl (pH 7.4), 1mM-EGTA, 1mM-dithiothreitol, fatty acid-poor albumin (20 or 80μM; see the Results and discussion section) and the indicated concentrations of palmitoyl-CoA and malonyl-CoA. The reaction was initiated by addition of 25μl containing 0.5μCi of L-[*Me*-^{3}H]-carnitine and the indicated concentration of L-carnitine. After 4min the reaction was terminated and treated as described by Saggerson *et al.* (1982).

Specific binding of [2-^{14}C]malonyl-CoA to mitochondria was measured as described by Bird & Saggerson (1984). Mitochondria were incubated with [2-^{14}C]malonyl-CoA at 0–4°C for 20min in the same medium as was used for assay of CPT_1 with 20μM-albumin. Constant binding is achieved within this time (Bird & Saggerson, 1984). Non-specific entrapment of [^{14}C]malonyl-CoA was taken as the radioactivity remaining bound in the presence of 0.5mM unlabelled malonyl-CoA and was subtracted from all values to give the amount of specifically bound malonyl-CoA. Computer analysis of malonyl-CoA binding data was performed as described by Bird & Saggerson (1984).

Statistical methods

Statistical significance was determined by Student's t test.

Results and discussion

Effect of malonyl-CoA on the K_m for carnitine

In preliminary experiments, the concentration of bovine serum albumin was 20μM and palmitoyl-CoA concentration was either 40μM or 10μM. To minimize changes in total palmitoyl-CoA concentration during the assay, which could introduce significant error, particularly when using low substrate concentrations, the albumin concentration in subsequent experiments was raised to 80μM, and palmitoyl-CoA concentration was increased proportionally. The absolute concentrations are stated in the legends to Tables and Figures. For consistency, results are expressed with respect to the molar concentration ratio of palmitoyl-CoA/albumin.

Tables 1 shows that when hepatic CPT_1 from fed rats was assayed with a palmitoyl-CoA/albumin molar ratio of 2.0, 10μM-malonyl-CoA did not significantly change the apparent K_m for carnitine, confirming previous findings (Saggerson & Carpenter, 1981*b*). With a lower ratio of palmitoyl-CoA/albumin (0.5), 10μM-malonyl-CoA significantly increased the apparent K_m for carnitine by 2.2-fold. This effect was seen in the fed, but not in the starved, state. In the fed state the percentage effect of malonyl-CoA on the '$V_{max.}$' (this $V_{max.}$ represents maximal activity at saturating carnitine concentrations, but is not the true $V_{max.}$, since [palmitoyl-CoA] cannot be saturating if inhibitory effects of malonyl-CoA are under investigation; Saggerson & Carpenter, 1981*c*) was independent of the palmitoyl-CoA/albumin molar ratio, in accord with previous findings (Saggerson & Carpenter, 1982*b*; Saggerson *et al.*, 1982). This result also suggests that in liver there may be two components to the malonyl-CoA-mediated inhibition of CPT_1 activity, namely a carnitine-dependent and a

Table 1. *Effect of malonyl-CoA on the kinetic parameters of CPT_1*

Liver CPT_1 was assayed with 40 μM-, 60 μM-, 80 μM-, 100 μM-, 150 μM-, 200 μM- and 400 μM-L-carnitine. Heart CPT_1 was assayed with 50 μM-, 100 μM, 200 μM-, 350 μM- and 500 μM-L-carnitine. The concentration of bovine serum albumin was 20 μM, and palmitoyl-CoA concentration was either 10 μM or 40 μM. K_m and '$V_{max.}$' values were determined from Lineweaver–Burk plots ($r \geqslant 0.98$ in every case). The values are means ± S.E.M. for the numbers of separate experiments indicated in parentheses in the first column. The values in brackets in the last column indicate the percentage decrease in '$V_{max.}$' caused by malonyl-CoA. * Indicates significant effects of malonyl-CoA ($P < 0.01$).

Tissue	Dietary state	Palmitoyl-CoA/ albumin molar ratio	Malonyl-CoA concn. (μM)	Apparent K_m for carnitine (μM)	'$V_{max.}$' (nmol/min per mg of protein)
Liver (6)	Fed	0.5	0	66 ± 6	1.29 ± 0.03
			10	144 ± 18*	0.77 ± 0.08* [−40 ± 6%]
Liver (5)	Fed	2.0	0	86 ± 15	5.47 ± 0.21
			10	98 ± 11	3.39 ± 0.27* [−38 ± 3%]
Liver (5)	Starved (24h)	0.5	0	68 ± 4	1.61 ± 0.15
			10	70 ± 3	1.22 ± 0.09* [−24 ± 3%]
Heart (4)	Fed	0.5	0	127 ± 22	2.46 ± 0.15
			0.2	139 ± 20	0.80 ± 0.18* [−68 ± 5%]

carnitine-independent component. The effect of malonyl-CoA on the K_m for carnitine was also investigated in heart mitochondria. Heart CPT_1 shows several differences in properties from the liver enzyme (Saggerson & Carpenter, 1981*b*; Saggerson, 1982; McGarry *et al.*, 1983; Mills *et al.*, 1983), including considerably greater sensitivity to malonyl-CoA. Table 1 shows that 0.2 μM-malonyl-CoA, which decreased '$V_{max.}$' by almost 70%, had no effect on the K_m for carnitine when the heart enzyme was assayed with a palmitoyl-CoA/albumin molar ratio of 0.5.

Previous work with a partially purified preparation of carnitine palmitoyltransferase has indicated that the kinetics of the reaction with respect to carnitine concentration are highly dependent on the concentration of the second substrate, palmitoyl-CoA (Bremer & Norum, 1967). It was proposed by Bremer & Norum (1967) that palmitoyl-CoA might affect the apparent K_m for carnitine by interacting at a site distinct from the acyl-CoA substrate-binding site. Whether these findings can be extrapolated to CPT_1 *in situ* in the mitochondrial membrane is less certain. Nevertheless, it is possible that in the experiments shown in Table 1 malonyl-CoA could indirectly affect the kinetics with respect to carnitine by competing with, and thereby decreasing, the binding of palmitoyl-CoA. This possibility was investigated by determining the apparent K_m of CPT_1 for carnitine over a wide range of palmitoyl-CoA concentrations in either the presence or the absence of 10 μM-malonyl-CoA (Fig. 1). Several conclusions can be drawn from this experiment. First, in the absence of malonyl-CoA, the apparent K_m for carnitine of CPT_1 *in situ* in the mitochondrial membrane was highly dependent on palmitoyl-CoA concentration, particularly

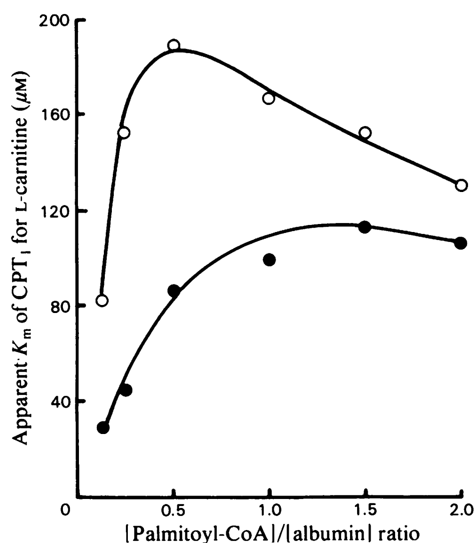

Fig. 1. *Influence of the palmitoyl-CoA/albumin ratio on the apparent K_m of CPT_1 for carnitine in the absence or presence of 10 μM-malonyl-CoA*

Liver CPT_1 from fed rats was assayed with 40 μM-, 60 μM-, 80 μM-, 100 μM-, 150 μM-, 200 μM- and 400 μM-L-carnitine. The concentration of albumin was 80 μM and palmitoyl-CoA concentration was 10, 20, 40, 80, 120 or 160 μM. K_m values were determined from Lineweaver–Burk plots ($r \geqslant 0.98$ in every case) and are the means of two similar experiments: ●, without malonyl-CoA; ○, with 10 μM-malonyl-CoA.

at the lower concentrations used. Second, in the absence of malonyl-CoA the estimated K_m values were always of the same order as, or lower than, the reported hepatic concentrations of carnitine

(McGarry *et al.*, 1975; Long *et al.*, 1982). Third, 10 μM-malonyl-CoA modified the effect of palmitoyl-CoA on carnitine kinetics in an unexpected way. Increasing the palmitoyl-CoA/albumin ratio from 0.125 to 0.5 caused an increase in the apparent K_m for carnitine, such that the K_m value was always 2.2–3.0-fold greater than at corresponding palmitoyl-CoA concentrations in the absence of malonyl-CoA. Under these conditions, the K_m for carnitine was raised above normal hepatic carnitine concentrations. However, in the presence of malonyl-CoA, increasing the palmitoyl-CoA/albumin ratio above 0.5 then caused a decrease in the apparent K_m for carnitine such that the effect of malonyl-CoA was minimal when the palmitoyl-CoA/albumin ratio reached 2.0. Fourth, the malonyl-CoA-induced increase in the apparent K_m for carnitine at low palmitoyl-CoA/albumin ratios cannot be explained by a direct competitive effect of malonyl-CoA versus palmitoyl-CoA.

In standard preparations of intact mitochondria, there is always a small proportion of CPT activity, routinely <15%, that is insensitive to malonyl-CoA (Saggerson & Carpenter, 1981*a*; Edwards *et al.*, 1985). This CPT activity is thought to be due to exposure of CPT_2 in damaged mitochondria. In the presence of 10 μM-malonyl-CoA, the percentage contribution of CPT_2 to measured CPT activity becomes greater as the palmitoyl-CoA/albumin ratio is decreased, and this contribution could affect the validity of the results shown in Fig. 1. Therefore, the following experiment was performed. CPT activity was measured in intact mitochondrial preparations in the presence of 0, 10 μM- or 100 μM-malonyl-CoA at each of the palmitoyl-CoA and carnitine concentrations shown in Fig. 1; 100 μM-malonyl-CoA was assumed to inhibit CPT_1 activity completely. In some experiments total CPT activity was also measured in sonicated (2 min at 0–5°C) mitochondria. The corrected activities of CPT_1 were taken therefore as: activity at zero malonyl-CoA minus activity at 100 μM-malonyl-CoA for the uninhibited enzyme, and activity at 10 μM-malonyl-CoA minus activity at 100 μM-malonyl-CoA for the enzyme inhibited by 10 μM-malonyl-CoA. The effect of malonyl-CoA on the apparent K_m of CPT_1 for carnitine at various palmitoyl-CoA/albumin ratios was qualitatively similar to that shown in Fig. 1, and we conclude that, when the acyl-CoA substrate concentration is low, malonyl-CoA can specifically alter the interaction of carnitine with the active site of hepatic CPT_1.

In the above experiment, CPT_2 activity was estimated as total CPT activity minus CPT_1 activity. The apparent K_m value of CPT_2 for carnitine was 250 μM and 415 μM at palmitoyl-CoA/albumin ratios of 0.5 and 2.0 respectively. Thus, as observed previously with brain non-synaptic mitochondrial CPT (Bird *et al.*, 1985), the apparent K_m of CPT_2 for carnitine is 3–4-fold greater than that for CPT_1. Although precise measurements are lacking, it has been shown that the mitochondrial carnitine concentration is positively correlated with liver carnitine, and the mitochondrial concentration has been estimated as 190 μM (Parvin & Pande, 1979). The physiological implication of the above findings is that a relatively high K_m of CPT_2 for carnitine will help to ensure flux of long-chain fatty-acyl groups into the mitochondrial matrix.

From the data shown in Table 1 and Fig. 1 it can be predicted that, at low palmitoyl-CoA/albumin ratios, the effectiveness of malonyl-CoA as an inhibitor of CPT_1 activity can be modified by the carnitine concentration in assays with liver mitochondria from fed but not from starved rats. This prediction was tested in the experiment shown in Table 2, which shows that the IC_{50} for malonyl-CoA in liver mitochondria of fed animals was 30% lower when assays were performed at 100 μM- rather than 400 μM-carnitine with a palmitoyl-

Table 2. *Influence of carnitine concentration on the inhibition of hepatic CPT_1 activity by malonyl-CoA*

Liver CPT_1 activity was assayed with 80 μM-albumin, 40 μM- or 160 μM-palmitoyl-CoA and 100 μM- or 400 μM-carnitine. Malonyl-CoA concentrations were 0, 0.25, 0.5, 0.75, 1, 2, 5, 10, 20 and 100 μM for assays with mitochondria from fed animals, and 0, 5, 10, 20, 30, 50, 100 and 200 μM for assays with mitochondria from starved rats. The IC_{50} values were read directly from graphs of percentage inhibition of CPT_1 activity versus [malonyl-CoA], and were corrected for malonyl-CoA-insensitive CPT. The values are means ± S.E.M. (where appropriate) for the numbers of separate experiments indicated in parentheses: *indicates a significant effect of carnitine (paired *t* test; $P<0.005$).

Dietary state	Palmitoyl-CoA/albumin ratio	[Carnitine] (μM)	IC_{50} for malonyl-CoA (μM)
Fed (3)	0.5	100	0.7 ± 0.1
		400	1.0 ± 0.1*
Starved (3)	0.5	100	15 ± 4
		400	17 ± 4
Fed (2)	2.0	100	9.8
		400	9.8

CoA/albumin ratio of 0.5. This effect was not observed with mitochondria from starved animals, nor when assays with mitochondria from fed animals were conducted with a palmitoyl-CoA/albumin ratio of 2.0. The data also show that at a palmitoyl-CoA/albumin ratio of 0.5 there was a 17-fold increase in the IC_{50} for malonyl-CoA on starvation. This contrasts with a 6-fold increase observed previously when assays were conducted in identical conditions but with a palmitoyl-CoA/albumin ratio of 2.0 (Saggerson & Carpenter, 1981*a*). This difference could be explained on kinetic grounds if malonyl-CoA introduces a greater sigmoidicity in the relationship between CPT_1 activity and palmitoyl-CoA concentration in hepatic mitochondria from fed rats than in those from starved animals (Saggerson & Carpenter, 1981*c*). Alternatively, if it assumed that the malonyl-CoA-binding site is distinct from the CPT_1 active site (Bird & Saggerson, 1984), then the 'fed–starved' sensitivity change in malonyl-CoA inhibition may be dependent, in part, on low concentrations of palmitoyl-CoA being more effective at decreasing the interaction between the malonyl-CoA-binding site and the CPT_1 active site in liver mitochondria from starved rats. It has previously been shown that starvation alters neither the binding characteristics of malonyl-CoA nor the competitive effects of palmitoyl-CoA on malonyl-CoA binding (Bird & Saggerson, 1984).

Binding of [^{14}C]*malonyl-CoA to intact mitochondria*

Binding studies were conducted firstly where malonyl-CoA and carnitine were the only ligands in the system. Fig. 2(*a*) shows that L-carnitine decreased the binding of 0.1 μM-[2-^{14}C]malonyl-CoA to liver mitochondria from fed and starved animals. In similar experiments with heart mitochondria L-carnitine was ineffective. The effect seen in liver mitochondria was stereospecific, since D-carnitine was ineffective. Fig. 2(*a*) shows that L-carnitine caused greater displacement of [^{14}C]-malonyl-CoA in the starved state, particularly at the lower carnitine concentrations. Maximal displacement and composite I_{50} values were calculated from several experiments and were found to be (means ± S.E.M.): in the fed state ($n = 4$), maximal displacement = 37 ± 1% and $I_{50} = 30 \pm 5\,\mu$M; in the starved state ($n = 7$), maximal displacement = 43 ± 1% ($P < 0.02$) and $I_{50} = 11 \pm 1\,\mu$M ($P < 0.001$). Reciprocal plots of these data (Fig. 2*b*) indicated that the displacement of [^{14}C]malonyl-CoA by L-carnitine was a complex process, suggesting interactions at more than one site. When total malonyl-CoA concentration in binding assays was 10 μM, the concentration-dependence of the displacement of bound malonyl-CoA on L-carnitine was qualitatively similar to that shown in

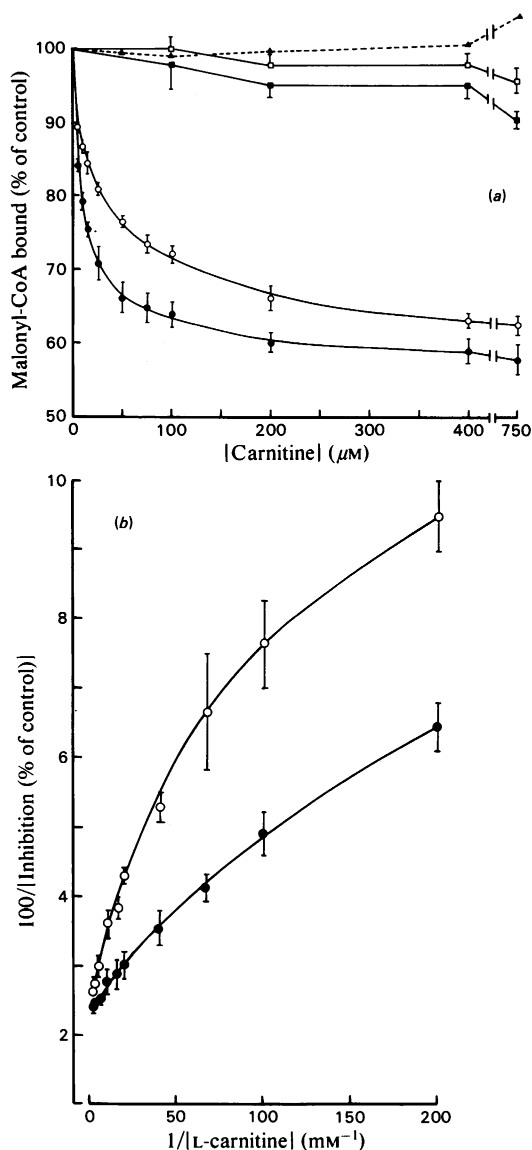

Fig. 2. *Effect of* L-*carnitine on* [^{14}C]*malonyl-CoA binding to liver and heart mitochondria*

Binding studies were carried out with 0.1 μM- (liver) or 0.05 μM- (heart) [2-^{14}C]malonyl-CoA and the indicated concentrations of D- or L-carnitine. (*a*) The values are expressed as percentages of binding in the absence of carnitine, which for liver mitochondria was 7.1 ± 0.5 and 5.2 ± 0.2 pmol/mg of protein in the fed and starved states respectively. For heart mitochondria, the amount of malonyl-CoA bound in the absence of carnitine was 7.4 ± 0.2 pmol/mg of protein. Effects of L-carnitine are shown by: ○, 'fed' liver; ●, 'starved' liver; ▲, 'fed' heart (means ± S.E.M. for three, five and three experiments respectively). Effects of D-carnitine are shown by □, 'fed' liver; ■, 'starved' liver (means ± S.E.M. for four experiments in both states). (*b*) Reciprocal plots of the data from (*a*); the symbols are the same.

Fig. 2(*a*), giving an I_{50} value of approx. 25μM-carnitine, but a maximal displacement of only 22% at 750μM-carnitine (mean values of two experiments with liver mitochondria from fed rats). Similar values were obtained in experiments with liver mitochondria from starved rats. Since L-carnitine bears little structural resemblance to malonyl-CoA, it is likely that in liver mitochondria the two compounds bind at independent, but interacting, sites. The nature of these binding sites is unclear, owing to the complexity of malonyl-CoA binding to intact mitochondrial membranes (Bird & Saggerson, 1984).

Fig. 3 shows that 400μM-carnitine (which causes approximately maximal displacement of bound malonyl-CoA; see above) decreased malonyl-CoA binding to liver mitochondria from fed animals over a wide range of [malonyl-CoA]. Scatchard plots of the binding data showed pronounced curvature, as observed previously (Bird & Saggerson, 1984), and were analysed according to a two-site model which may or may not be adequate for this complex binding profile. The calculated binding parameters are shown in the legend to Fig. 3, and indicate that the major effect of carnitine was to decrease the maximal binding capacity at high-affinity sites (N_1) by 35%. Three experiments similar to that shown in Fig. 3 were performed with mitochondria from starved animals (results not shown). The results were qualitatively similar, and the most noteworthy findings were that carnitine decreased N_1 from 4.8 ± 0.3 to 2.7 ± 0.3 pmol/mg of protein ($P < 0.01$) without significantly changing the other binding parameters. It is evident from the above experiments that a large proportion of bound malonyl-CoA cannot be displaced from its binding sites even at carnitine concentrations approaching saturation. This observation may be compared with the previous finding that the inhibition of CPT_1 activity by malonyl-CoA in the fed state can only be partially overcome by carnitine (Table 1).

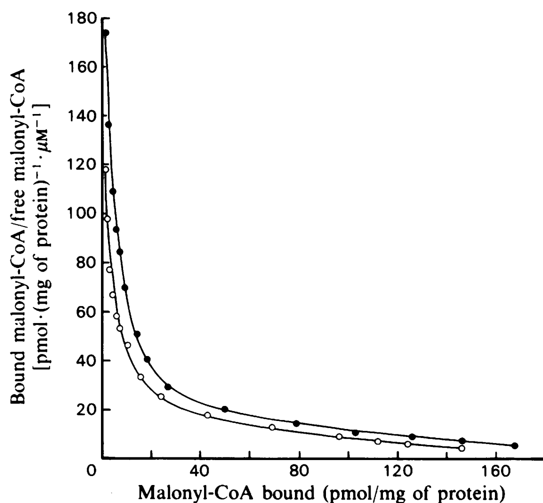

Fig. 3. *Effect of L-carnitine on [¹⁴C]malonyl-CoA binding to liver mitochondria from fed animals*

Binding was measured at 15 different concentrations of [2-¹⁴C]malonyl-CoA (0.01–30μM). The values are means for three experiments and are presented in the form of a Scatchard plot. The data from the individual experiments were analysed by using a two-site model. In the absence of carnitine, this showed: $K_{D(1)} = 40 \pm 9$ nM; $K_{D(2)} = 4.8 \pm 0.8$ μM; $N_1 = 6.5 \pm 0.9$ pmol/mg of protein; $N_2 = 144 \pm 25$ pmol/mg of protein. In the presence of carnitine, these values were: $K_{D(1)} = 40 \pm 4$ nM; $K_{D(2)} = 4.6 \pm 0.8$ μM; $N_1 = 4.2 \pm 0.8$ pmol/mg of protein; $N_2 = 128 \pm 14$ pmol/mg of protein. ●, Without carnitine; ○, with L-carnitine (400μM).

In order to observe the interacting actions of carnitine and malonyl-CoA under CPT_1 assay conditions, the fatty acyl-CoA substrate must obviously be present. Therefore the concentration-dependence of the displacement of 10μM-malonyl-CoA on carnitine was also studied in standard binding assays in the presence of 10μM-palmitoyl-CoA (palmitoyl-CoA/albumin ratio 0.5). This concentration of palmitoyl-CoA alone caused a 16% decrease in the amount of malonyl-CoA bound (cf. Bird & Saggerson, 1984), but an additional concentration-dependent decrease in binding was observed in the presence of carnitine (results not shown). Some of these results are summarized in Table 3, which shows that 400μM-carnitine and 10μM-palmitoyl-CoA were equally effective in displacing bound malonyl-CoA, but that the displacement caused by the two substrates together was only partially additive. Similar results were obtained with liver mitochondria from both fed and starved rats. The possibility of a substantial loss of palmitoyl-CoA through the CPT_1 reaction was investigated by including 0.5μCi of [³H]carnitine (total carnitine concentration of 400μM) in the standard binding assay (20min at 0–5°C). It was found that approx. 25% of the palmitoyl-CoA was converted into palmitoylcarnitine during the assay. In a separate experiment, it was found that 10μM-palmitoylcarnitine did not decrease 10μM-malonyl-CoA binding, but that 20μM-CoA decreased binding by about 50% (mean value for two experiments with liver mitochondria from starved rats).

Table 3 also confirms that L-carnitine had no effect on malonyl-CoA binding in heart mitochondria, but that carnitine can partially overcome the displacing effect of palmitoyl-CoA. This result is in contrast with the synergistic effect of L-carnitine

Table 3. *Effects of palmitoyl-CoA and carnitine on malonyl-CoA binding to isolated liver and heart mitochondria*
Intact mitochondria were incubated for 20min at 0–4°C with 10μM-[^{14}C]malonyl-CoA (liver) or 0.2μM-[^{14}C]-malonyl-CoA (heart) and the additions indicated below. The concentrations of L-carnitine, palmitoyl-CoA and albumin were 400μM, 10μM and 20μM respectively. Values are the means ± S.E.M. for five (liver, fed and starved) or three (heart) separate experiments, and results are expressed as a percentage of the amount of malonyl-CoA bound in the absence of other additions, which was 102 ± 10, 96 ± 9 and 10.1 ± 0.2pmol/mg of protein for mitochondria from 'fed' liver, 'starved' liver and 'fed' heart respectively.

	Malonyl-CoA bound (% of control)		
	Liver		Heart
Additions	Fed	Starved	Fed
None	100	100	100
L-Carnitine	80.6 ± 1.2	84.4 ± 1.5	109 ± 5
Palmitoyl-CoA	83.6 ± 4.3	83.4 ± 1.6	79 ± 4
Palmitoyl-CoA + L-carnitine	74.8 ± 1.6	75.6 ± 1.6	89 ± 7

and either 2-bromoacetyl-CoA or 2-bromopalmitoyl-CoA on malonyl-CoA binding to heart mitochondria (see accompanying paper, Edwards *et al.*, 1985).

General conclusions

This study establishes that at low palmitoyl-CoA/albumin ratios L-carnitine and malonyl-CoA can specifically interact in the hepatic CPT_1 system. On the one hand, malonyl-CoA diminishes the effectiveness of carnitine as a substrate for CPT_1. On the other hand, carnitine has effects which might diminish the regulatory effect of malonyl-CoA. The physiological implication of these findings in fed animals is that, by raising the K_m of CPT_1 for carnitine above physiological concentrations of this compound, malonyl-CoA decreases CPT_1 activity to a greater extent than expected by direct inhibition of the enzyme alone. In conditions where hepatic carnitine concentrations are acutely increased (e.g. after glucagon or anti-insulin serum administration; McGarry *et al.*, 1975), malonyl-CoA inhibition of CPT_1 activity could be partially overcome. For reasons not at present understood, these effects are less pronounced in mitochondria from starved animals, or when the concentration of palmitoyl-CoA is increased. The physical nature of the sites of these interactions and the nature of the starvation-induced change have yet to be established.

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