Effect of membrane environment on the activity and inhibitability by malonyl-CoA of the carnitine acyltransferase of hepatic microsomal membranes

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We have investigated the extent to which membrane environment affects the catalytic properties of the malonyl-CoA-sensitive carnitine acyltransferase of liver microsomal membranes. Arrhenius-type plots of activity were linear in the absence and presence of malonyl-CoA (2.5 μ M). Sensitivity to malonyl-CoA increased with decreasing assay temperature. Partly purified enzyme displayed an increased $K_{0.5}$ (substrate concentration supporting half the maximal reaction rate) for myristoyl-CoA and a reduced sensitivity to malonyl-CoA compared with the enzyme *in situ* in membranes. Reconstitution with liposomes of a range of compositions restored the $K_{0.5}$ for myristoyl-CoA to values similar to that seen in native membranes. The lipid requirements for restoration of sensitivity to malonyl-CoA were more stringent. When animals were starved for 24 h the specific activity of carnitine acyltransferase in microsomal membrane

residues was increased 3.3-fold, whereas sensitivity to malonyl-CoA was decreased to 1/2.8. When enzymes partly purified from fed and starved animals were reconstituted into crude soybean phosphatidylcholine liposomes there was no difference in sensitivity to malonyl-CoA. When partly purified enzyme from fed rats was reconstituted into liposomes prepared from microsomal membrane lipids from fed animals it was 2.2-fold more sensitive to malonyl-CoA than when reconstituted with liposomes prepared from microsomal membrane lipids from starved animals. This suggests that the physiological changes in sensitivity to malonyl-CoA are mediated via changes in membrane lipid composition rather than via modification of the enzyme protein itself. The increased specific activity of acyltransferase observed on starvation could not be attributed to changes in membrane lipid composition.

INTRODUCTION

Tightly membrane-bound carnitine acyltransferases (CATs) that are potently inhibited by physiological concentrations of malonyl-CoA have been described in mitochondria, microsomes and peroxisomes [1–6]. Carnitine palmitoyltransferase of the mitochondrial outer membrane (CPT₁) is, by virtue of its inhibitability by malonyl-CoA, a key regulatory site controlling the flux through β -oxidation [7,8]. Hepatic CPT₁ displays increased specific activity and decreased sensitivity to malonyl-CoA in starvation and diabetes [9–14]. Consequently much attention has been directed at understanding the mechanisms underlying these changes. The increased activity is due to an increased abundance of CPT₁ protein [15,16], regulated at the transcriptional level [16,17]. The mechanisms underlying the changes in sensitivity to malonyl-CoA are less well understood. A number of laboratories, using a range of approaches, have suggested that the inhibition of CPT₁ by malonyl-CoA is strongly influenced by membrane environment [18–25]. However, a major problem in studying the effects of membrane environment on the properties of CPT₁ is the inability to solubilize the enzyme in a stable, active state [6,19,26,27], which is a prerequisite for reconstitution in a defined lipid environment. Thus although the lipid composition of the mitochondrial outer membrane, in which CPT₁ resides, has been shown to change with physiological state [23], it has not been possible to show convincingly that the physiological changes in sensitivity to malonyl-CoA are a direct result of these changes in membrane composition. Recently it has become apparent that the CATs of peroxisomes and microsomes also display increased specific activity and decreased sensitivity to malonyl-CoA on starvation [17,28,29]. To investigate more

directly the role of the membrane in modulating the properties of the malonyl-CoA-sensitive CATs we have used, as a model system, the transferase of liver microsomal membranes. This enzyme can be solubilized in good yield with excellent long-term stability [6] and has been partly characterized [6,30]. Here we report on the use of this system to study the lipid requirements for catalytic function and inhibition by malonyl-CoA by reconstituting partly purified enzyme in liposomes of various lipid compositions. We provide the first direct demonstration that the different sensitivity to inhibition by malonyl-CoA seen in different physiological states is indeed mediated via changes in microsomal membrane lipid composition.

MATERIALS AND METHODS

Chemicals

Crude soybean phosphatidylcholine (PC; product no. P3644, approx. 40%), pure soybean PC (99%), soybean phosphatidylethanolamine (PE; 98%), soybean phosphatidylinositol (PI; 98%), bovine brain phosphatidylserine (PS; 98%), triolein (99%), chicken egg yolk sphingomyelin (99%) and cholesterol (99%) were from Sigma. All other reagents were as described previously [6].

Animals

Male Sprague–Dawley rats (approx. 200–250 g) were used throughout. Fed animals were maintained as described previously [6]. Starved animals were treated in an identical manner except that food, but not water, was withdrawn at 10:00h, 24 h before they were killed.

Abbreviations used: CAT, carnitine acyltransferase; CPT₁, carnitine palmitoyltransferase of the mitochondrial outer membrane; $K_{0.5}$, concentration supporting half the maximal reaction rate; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine; TAG, triacylglycerol.

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Isolation of microsomal membranes

Rough microsomal membranes were isolated from liver by sucrose-density gradient centrifugation [31]. Microsomal membrane residues, depleted of malonyl-CoA-insensitive CAT, were prepared by freeze-thawing and ultracentrifugation to release the malonyl-CoA-insensitive enzyme as described previously [6].

CAT

Malonyl-CoA-sensitive CAT was solubilized from microsomal membrane residues with sodium deoxycholate, and partly purified by gel filtration on Superdex 200 [6].

Assay of CAT

CAT was assayed spectrophotometrically in the direction of acylcarnitine formation. Unless stated otherwise the assay temperature was 25 °C. Assay mixtures (1 ml) contained: 50 mM $\text{KH}_2\text{PO}_4/\text{K}_2\text{HPO}_4$ buffer (pH 7.5), 1.3 mg/ml fatty acid-poor BSA, fatty acyl-CoA (50 μM when measuring IC_{50} values; 100 μM when determining maximal rates), 125 μM 4,4'-dithiodipyridine, 20–100 μg of sample protein and 0–50 μM malonyl-CoA; assays were initiated by the addition of 2 mM L-carnitine. Rates were corrected for carnitine-independent CoA release. Activity was calculated by using $\epsilon_{324} = 19.4 \text{ mM}^{-1} \cdot \text{cm}^{-1}$.

Extraction of microsomal lipids

Microsomal lipids were extracted with chloroform/methanol exactly as described by Bligh and Dyer [32]. The final chloroform extract was evaporated to dryness under a nitrogen stream and stored under nitrogen at -70°C .

Reconstitution of CAT into liposomes

To form liposomes, stock solutions of lipids in chloroform were mixed in the appropriate ratios and then evaporated to dryness under nitrogen. The dried lipids were resuspended in distilled water at a concentration of 100 mg dry weight/ml by vortex mixing. Small unilamellar liposomes were formed by sonicating the lipid suspension to clarity on ice under nitrogen with the microtip of an MSE Soniprep 150 probe sonicator. Malonyl-CoA-sensitive CAT from the Superdex 200 column was reconstituted with the preformed liposomes as described previously [6]. In all reconstitutions the lipid-to-protein ratio was approx. 40:1 (w/w).

Protein concentrations

Protein was determined with a bicinchoninic acid kit (Sigma) with fatty acid-poor BSA as standard.

Data analysis

Statistical significance was determined with Student's *t*-test for paired or unpaired samples as appropriate. Values are presented as the means $\pm$ S.E.M. for the stated number of separate measurements (*n*). Where S.E.M. bars are not visible they lie within the symbol.

RESULTS AND DISCUSSION

All microsomal membranes used in the present work were depleted of the loosely membrane-associated, malonyl-CoA-insensitive CAT by freeze-thawing and ultracentrifugation [6], and are referred to as microsomal membrane residues.

Figure 1 Fatty acyl-CoA substrate dependence of malonyl-CoA-sensitive CAT of microsomal membranes

Microsomal membrane residues depleted of malonyl-CoA-insensitive acyltransferase were isolated from fed animals. Activity was assayed at 25 °C in 50 mM potassium phosphate buffer (pH 7.5) containing 1.3 mg/ml albumin; 100% represents the activity measured at 100 μM fatty acyl-CoA in the absence of malonyl-CoA. Upper panel, decanoyl-CoA as substrate (100% corresponds to $14.2 \pm 0.5 \text{ nmol/min per mg of protein}$, $n = 3$ in each case); lower panel, myristoyl-CoA as substrate (100% corresponds to $9.5 \pm 0.8 \text{ nmol/min per mg of protein}$, $n = 4$ in each case). Symbols: $\circ$, without malonyl-CoA; $\bullet$, plus malonyl-CoA (5 μM).

Acyl-CoA assay substrate

In previous work we assayed microsomal CAT with decanoyl-CoA as substrate [6,30,33]. In the present work we have used myristoyl-CoA as substrate because preliminary experiments indicated that the malonyl-CoA-sensitive CAT of microsomes is more sensitive to changes in membrane environment when assayed with the longer chain-length substrate (this is discussed in detail below). Owing to the complex binding of fatty acyl-CoA to albumin [34], the free myristoyl-CoA concentration for a given total (i.e. bound plus free) concentration of myristoyl-CoA is unknown and thus it is not possible to determine true K_m values. However, kinetic parameters were always determined under identical conditions so that relative values of $K_{0.5}$ (substrate concentration supporting half the maximal reaction rate) for myristoyl-CoA and IC_{50} for malonyl-CoA after different treatments can be meaningfully compared without knowing the free myristoyl-CoA concentration. Malonyl-CoA displayed mixed-type inhibition with respect to decanoyl-CoA [6] (Figure 1, upper panel). In contrast, malonyl-CoA displayed competitive-like inhibition with respect to myristoyl-CoA (Figure 1, lower panel). Similar differences in the kinetics of inhibition by malonyl-CoA

Figure 2 Temperature dependence of malonyl-CoA-sensitive CAT of microsomal membranes

Membranes were as described in the legend to Figure 1. Activity was assayed at 50 μ M myristoyl-CoA over the temperature range 5–40 °C. Symbols: ○, without malonyl-CoA; ●, plus malonyl-CoA (2.5 μ M). The values are means $\pm$ S.E.M. for three separate experiments. In the absence of malonyl-CoA the mean slope was -0.768 (range -0.748 to -0.786). In the presence of malonyl-CoA the mean slope was -1.080 (range -0.949 to -1.181). Linear correlation coefficients for each of the separate experiments were never less than -0.992 and for the mean data were -0.997 in the absence of malonyl-CoA and -0.999 in the presence of malonyl-CoA. Slopes obtained in the presence of malonyl-CoA were significantly different from those obtained in the absence ($P < 0.001$ for the mean data as shown, and $P < 0.001$, $P < 0.001$ and $P < 0.01$ for each of the three individual experiments).

have been observed for CPT₁ assayed with octanoyl-CoA and palmitoyl-CoA [35]. As malonyl-CoA displayed competitive-like inhibition with respect to myristoyl-CoA, a myristoyl-CoA concentration of 50 μ M was used when measuring IC₅₀ values for malonyl-CoA inhibition.

Effect of assay temperature on CAT

Increased assay temperature is associated with an increase in membrane fluidity [14,36,37]. Increased membrane fluidity, whether induced by increased assay temperature or by chemical agents, has been shown previously to result in a decreased sensitivity of mitochondrial CPT₁ to inhibition by malonyl-CoA [20,23,25]. Similarly, in preliminary experiments it was observed that CAT of microsomal membrane residues was less sensitive to malonyl-CoA at 37 °C (IC₅₀ 17.5 ± 3.3 μ M, $n = 3$) than at 25 °C (IC₅₀ 1.8 ± 0.4 μ M, $n = 3$; $P < 0.01$ compared with 37 °C). It is possible that these changes in sensitivity to malonyl-CoA were only apparent and could have resulted from a temperature-dependent change in the $K_{0.5}$ for myristoyl-CoA. Alternatively temperature might affect the binding of myristoyl-CoA to albumin and hence the free concentration of myristoyl-CoA in CAT assays. However, in our standard assay, the dependence of CAT activity on myristoyl-CoA concentration was the same at 20 °C and at 37 °C (results not shown), suggesting that these reservations are unfounded. It is noteworthy that other workers have also found the fatty acyl-CoA concentration dependence of mitochondrial CAT activity to be independent of assay temperature [14]. We further investigated the effects of temperature-induced changes in membrane fluidity by assaying CAT over a range of temperatures from 5 to 40 °C in the absence and in the presence of a fixed concentration of malonyl-CoA (2.5 μ M). Arrhenius-type plots of these data (Figure 2) show a significantly ($P < 0.001$) greater slope in the presence than in the absence of malonyl-CoA. This experiment clearly demonstrates that as the

Figure 3 Fatty acyl-CoA substrate dependence of malonyl-CoA-sensitive microsomal CAT preparations

All preparations were from fed animals. Activity was assayed as described in Figure 1 with myristoyl-CoA as substrate. Assays of partly purified CAT (before and after reconstitution) contained additionally 0.25 mM sodium deoxycholate (owing to carry-over). After reconstitution, assays also contained 0.8 mg of liposome lipids. A value of 100% represents the activity at 100 μ M myristoyl-CoA. Symbols: ○, microsomal membrane residues depleted of malonyl-CoA-insensitive acyltransferase; ■, partly purified acyltransferase from the Superdex 200 column; ●, partly purified acyltransferase reconstituted in crude soybean PC liposomes; □, partly purified acyltransferase reconstituted in fed microsomal lipid liposomes; △, partly purified acyltransferase reconstituted in PC/PE/PI/PS liposomes. In each case $n = 3$ except for microsomal membrane residues, where $n = 4$.

assay temperature is increased, with a concomitant increase in the fluidity of the microsomal membrane, the degree of inhibition by a fixed concentration of malonyl-CoA (i.e. the sensitivity to inhibition by malonyl-CoA) is markedly attenuated. It is noteworthy that the Arrhenius-type plots are monophasic both in the absence and in the presence of malonyl-CoA. This is in marked contrast with hepatic mitochondrial outer membrane CPT₁, for which Arrhenius-type plots of both activity [14,25] and K_i for malonyl-CoA [25] are clearly biphasic over this temperature range. As biphasic Arrhenius plots have been observed for a number of enzymes of the microsomal membrane (e.g. *o*-demethylase activity [38], 3-hydroxy-3-methylglutaryl-CoA reductase [39] and corticosteroid 11-reductase [40]) over this same temperature range it is difficult to ascribe the different forms of the Arrhenius-type plots for the mitochondrial and microsomal enzymes simply to differences in the membranes themselves. Thus the different temperature dependences of the mitochondrial and microsomal CATs might reflect differences in their degrees of interaction with their respective membranes. This would be consistent with the observation that CPT₁ is more tightly bound to the membrane than is microsomal CAT. Concentrations of sodium cholate that fail to remove CPT₁ from the mitochondrial outer membrane (but do not inactivate the enzyme) [21,25] readily solubilize the microsomal malonyl-CoA-sensitive acyltransferase (N. M. Broadway and E. D. Saggerson, unpublished work). The key observation of the temperature-dependence experiment is that, as has been shown for mitochondrial CPT₁ [20,23,25], the fluidity of the membrane is an important factor in determining sensitivity to inhibition by malonyl-CoA.

Lipid requirements of microsomal malonyl-CoA-inhibitable CAT

Partly purified CAT was reconstituted with liposomes [6] of various lipid compositions (Figures 3 and 4, and Table 1). When myristoyl-CoA was used as substrate, solubilization with sodium

Figure 4 Effect of malonyl-CoA on microsomal CAT preparations

Assay conditions were as for Figure 3 except that myristoyl-CoA concentration was fixed at 50 μ M; $n = 3$ in each case. Symbols: $\circ$, microsomal membrane residues depleted of malonyl-CoA-insensitive acyltransferase; $\blacksquare$, partly purified acyltransferase from the Superdex 200 column; $\bullet$, partly purified acyltransferase reconstituted in crude soybean PC liposomes; $\square$, partly purified acyltransferase reconstituted in fed microsomal lipid liposomes; $\triangle$, partly purified acyltransferase reconstituted in PC/PE/PI/PS liposomes.

Table 1 Effect of reconstitution on maximal activity of partly purified malonyl-CoA-inhibitable CAT from fed and starved rats

Activities were measured at 100 μ M myristoyl-CoA. Activity before reconstitution was 8.9 ± 0.8 nmol/min per mg of protein ($n = 10$) for enzyme partly purified from fed animals and 29.0 ± 4.8 ($n = 3$) for enzyme partly purified from starved animals. Activation was calculated as (CAT activity in liposomes)/(CAT activity before reconstitution). Statistical significances: * $P < 0.01$ compared with Expts. 4 and 5, and $P < 0.001$ compared with Expt. 6; † $P < 0.01$ compared with Expts. 4 and 6, and $P < 0.02$ compared with Expt. 5; ‡significantly different from 1.00 ($P < 0.05$). Abbreviation: m.m.r., microsomal membrane residues.

Experiment	Enzyme	Liposomes	Activation (fold)	n
1	Fed	Fed m.m.r. lipid	$1.63 \pm 0.11^{*†‡}$	6
2	Fed	Starved m.m.r. lipid	$1.63 \pm 0.11^{*†‡}$	5
3	Fed	Crude soybean PC	$1.35 \pm 0.07^{*†}$	6
4	Fed	Pure soybean PC	0.96 ± 0.09	5
5	Fed	PC/PE/PI/PS	0.95 ± 0.12	3
6	Fed	PC/TAG	0.78 ± 0.09	3
7	Starved	Crude soybean PC	1.42 ± 0.11	3

deoxycholate resulted in a marked increase in the $K_{0.5}$ for the fatty acyl-CoA substrate (Figure 3) and a much decreased inhibition by malonyl-CoA (Figure 4). This is in contrast with the situation when the enzyme is assayed with decanoyl-CoA, in which case only a reduced sensitivity to inhibition by malonyl-CoA is observed without any change in the $K_{0.5}$ for the fatty acyl-CoA [6]. Thus the use of myristoyl-CoA as substrate allows the probing of the effects of membrane environment both on the basal kinetics of the enzyme (i.e. $K_{0.5}$ for fatty acyl-CoA) and on sensitivity to inhibition by malonyl-CoA in order to determine whether these two aspects of enzyme function have the same or different lipid requirements.

It seems that there are three aspects to the lipid requirements of microsomal CAT: an effect on $K_{0.5}$ for myristoyl-CoA, an effect on sensitivity to inhibition by malonyl-CoA, and the extent to which different lipids alter $V_{\max}$.

When partly purified CAT [6] was reconstituted with liposomes prepared from crude soybean PC, with lipids extracted from

microsomal membrane residues isolated from fed animals or with PC/PE/PI/PS (58:17:9:4, by wt.) the $K_{0.5}$ for myristoyl-CoA was restored to a value essentially identical with that before solubilization (Figure 3). Similar results were obtained when using liposomes prepared from pure soybean PC and pure PC/triacylglycerol (PC/TAG; 90:10, w/w) (results not shown). Thus the lipid requirements of this aspect of catalytic function are not particularly specific. The same lipid mixtures had rather different effects on the IC_{50} for malonyl-CoA (Figure 4). Reconstitution with crude soybean PC or with lipids from fed microsomal membrane residues substantially increased the sensitivity to inhibition by malonyl-CoA compared with CAT before reconstitution. In contrast, reconstitution with PC/PE/PI/PS liposomes was far less effective at restoring malonyl-CoA sensitivity. Indeed, at approximately physiological concentrations of malonyl-CoA (up to 10 μ M) the enzyme reconstituted with PC/PE/PI/PS liposomes was not appreciably more sensitive to inhibition compared with enzyme before reconstitution; only at high concentrations of malonyl-CoA was the degree of inhibition greater than for the enzyme before reconstitution. A similar pattern was seen with pure PC and with pure PC/TAG (90:10, w/w) (results not shown), i.e. restoration of the normal $K_{0.5}$ for myristoyl-CoA was seen but with little effect on malonyl-CoA inhibibility. It should be noted that reconstitution into liposomes prepared from pure PC/sphingomyelin (90:10, w/w) or pure PC/cholesterol (90:10, w/w) also had little effect on sensitivity to malonyl-CoA (results not shown). In these latter cases the effect on $K_{0.5}$ for myristoyl-CoA was not examined.

We further investigated the lipid requirements of microsomal CAT by examining the activity at saturating myristoyl-CoA concentration in a number of lipid environments (Table 1). A different pattern emerges here from that seen for the effect on $K_{0.5}$ for myristoyl-CoA. Reconstitution with microsomal membrane lipids and crude soybean PC resulted in a significant ($P < 0.05$) increase in $V_{\max}$. Pure soybean PC and PC/PE/PI/PS had no effect on $V_{\max}$, whereas pure PC/TAG produced a small, but not significant, decrease in $V_{\max}$. Clearly the lipid requirements for an increase in $V_{\max}$ are quite stringent and are different from those for inhibition by malonyl-CoA. Microsomal lipids support a higher $V_{\max}$ than crude soybean PC but crude soybean lipids confer greater sensitivity to malonyl-CoA (Table 1 and Figure 4). The component(s) of the complex lipid mixtures responsible for activation and malonyl-CoA-sensitivity have not been identified. However, it seems that none of the major lipid species of the microsomal membrane (e.g. PC, PE, PI, PS, sphingomyelin or cholesterol) fulfils this role.

Lipids mediate physiological changes in CAT

When animals were starved for 24 h, CAT specific activity (assayed at 100 μ M myristoyl-CoA) in microsomal membrane residues was increased 3.3-fold from 10.0 ± 0.9 nmol/min per mg of protein ($n = 8$) in the fed state to 33.1 ± 3.5 nmol/min per mg of protein ($n = 6$, $P < 0.001$ compared with fed). The IC_{50} for malonyl-CoA was increased by 2.8-fold on starvation to 5.0 ± 0.8 μ M ($n = 4$) from 1.8 ± 0.4 μ M ($n = 3$, $P < 0.02$ compared with starved) in the fed state. Similar findings have been reported by other laboratories working with intact microsomal vesicles [17,29]. Given that inhibition by malonyl-CoA is competitive with respect to myristoyl-CoA (Figure 1, lower panel), an increase in IC_{50} could be brought about by a decrease in the $K_{0.5}$ for myristoyl-CoA, which would result in an increased degree of saturation by the 50 μ M myristoyl-CoA substrate. However, the $K_{0.5}$ for myristoyl-CoA was unaltered on starvation (results not shown), indicating that the increased IC_{50} observed

under these conditions does genuinely represent a decreased sensitivity to inhibition by malonyl-CoA. In view of the influence of lipid environment on microsomal CAT it was decided to investigate whether the changes in CAT observed on starvation could be attributed to changes in the microsomal membrane lipids. Malonyl-CoA-inhibitable CAT was partly purified from microsomal membrane residues isolated from 24 h-starved animals and then reconstituted with crude soybean PC liposomes. The increased CAT specific activity observed in starved membranes was retained through solubilization and partial purification (see legend to Table 1). When partly purified CAT from starved microsomal membrane residues was reconstituted in crude soybean PC liposomes it was activated 1.42-fold, which is essentially identical with the 1.35-fold activation obtained with partly purified CAT derived from fed microsomal membrane residues (Table 1). The difference in IC_{50} values for malonyl-CoA between CAT in fed membranes and CAT in starved membranes (Figure 5, top panel) disappeared when the corresponding partly purified CATs were reconstituted into identical (crude soybean PC) membrane environments (Figure 5, middle panel). When partly purified CAT from fed microsomal membranes was reconstituted into liposomes prepared from lipids from starved microsomal membrane residues, it was 1/2.2 as sensitive to inhibition by malonyl-CoA ($IC_{50} = 8.7 \pm 1.5 \mu M$, $n = 6$) than when reconstituted into liposomes prepared from lipids from fed microsomal membrane residues ($IC_{50} = 4.0 \pm 0.7 \mu M$, $n = 6$, $P < 0.02$ compared with starved lipids) (Figure 5, bottom panel). It is noteworthy that this difference in sensitivity to malonyl-CoA seen in the reconstituted system (2.2-fold) is similar to the difference in malonyl-CoA-sensitivity between CAT in native fed membranes and native starved membranes (2.8-fold). Together these results strongly suggest that different sensitivities to malonyl-CoA observed for CAT in membranes from fed and starved animals are due to changes in membrane lipid composition. The observation that CATs from fed and starved animals display the same sensitivities to malonyl-CoA and the same degrees of activation when reconstituted into identical lipid environments dispels the notion that the different sensitivities seen in native membranes are due to modification of the enzyme protein itself. This observation also dispels the notion of an auxiliary factor in the membrane of starved animals acting to decrease sensitivity to malonyl-CoA (or of a factor in fed membranes acting to increase sensitivity to malonyl-CoA). This is the first direct demonstration that the physiological changes in sensitivity to malonyl-CoA of a membrane-bound CAT [9–14,17,28,29] are indeed mediated via changes in membrane lipid composition.

CAT was activated to the same extent by both starved microsomal membrane lipids and fed microsomal membrane lipids (Table 1). Thus the 3.3-fold increase in acyltransferase specific activity seen on starvation cannot be attributed to changes in microsomal membrane lipid composition. It is likely, by analogy with mitochondrial CPT_1 , that the increased specific activity is due to increased abundance of enzyme protein. The complex lipid requirements of microsomal CAT with respect to sensitivity to malonyl-CoA presumably allow subtle remodelling of the lipid composition of the microsomal membrane in different physiological states to mediate substantial changes in sensitivity to this metabolite. Such remodelling of membrane lipids could potentially modulate sensitivity to malonyl-CoA via changes in membrane fluidity and/or changes in specific protein–lipid interactions. However, the precise mechanisms remain to be elucidated. The observation that the $K_{0.5}$ for myristoyl-CoA has a less stringent lipid requirement would explain why this parameter is unaffected by the same changes in the membrane. The

Figure 5 Malonyl-CoA inhibition of microsomal CAT in different physiological states

Activity was assayed as described in Figure 4. Top panel: microsomal membrane residues depleted of malonyl-CoA-insensitive acyltransferase were isolated from fed (●, $n = 3$) and from starved (○, $n = 4$) animals. Middle panel: partly purified CAT from fed animals (●, $n = 3$) and from starved animals (○, $n = 3$) was reconstituted with crude soybean PC liposomes. Bottom panel: partly purified CAT from fed animals was reconstituted with microsomal membrane lipids isolated from microsomal membranes obtained from fed (●, $n = 6$) and starved (○, $n = 6$) animals. In other experiments (results not shown) it was found that the maximal degree of inhibition was obtained with 50 μM malonyl-CoA.

microsomal malonyl-CoA-inhibitable CAT, despite being a distinct protein [5,6], is functionally very similar to mitochondrial outer-membrane CPT_1 . Thus information gained regarding the the lipid-mediated regulation of the microsomal enzyme is likely

also to provide insight into the mechanisms of lipid-mediated regulation of mitochondrial CPT₁.

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