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Observations on the affinity for carnitine, and malonyl-CoA sensitivity, of carnitine palmitoyltransferase I in animal and human tissues

Demonstration of the presence of malonyl-CoA in non-hepatic tissues of the rat

J. Denis McGARRY,* Scott E. MILLS, Carlin S. LONG and Daniel W. FOSTER
Departments of Internal Medicine and Biochemistry, University of Texas Health Science Center at Dallas, Dallas, TX 75235, U.S.A.

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The requirement for carnitine and the malonyl-CoA sensitivity of carnitine palmitoyltransferase I (EC 2.3.1.21) were measured in isolated mitochondria from eight tissues of animal or human origin using fixed concentrations of palmitoyl-CoA (50 μM) and albumin (147 μM). The K_m for carnitine spanned a 20-fold range, rising from about 35 μM in adult rat and human foetal liver to 700 μM in dog heart. Intermediate values of increasing magnitude were found for rat heart, guinea pig liver and skeletal muscle of rat, dog and man. Conversely, the concentration of malonyl-CoA required for 50% suppression of enzyme activity fell from the region of 2–3 μM in human and rat liver to only 20 nM in tissues displaying the highest K_m for carnitine. Thus, the requirement for carnitine and sensitivity to malonyl-CoA appeared to be inversely related. The K_m of carnitine palmitoyltransferase I for palmitoyl-CoA was similar in tissues showing large differences in requirement for carnitine. Other experiments established that, in addition to liver, heart and skeletal muscle of fed rats contain significant quantities of malonyl-CoA and that in all three tissues the level falls with starvation. Although its intracellular location in heart and skeletal muscle is not known, the possibility is raised that malonyl-CoA (or a related compound) could, under certain circumstances, interact with carnitine palmitoyltransferase I in non-hepatic tissues and thereby exert control over long chain fatty acid oxidation.

Recent studies with crude homogenates (Long *et al.*, 1982) revealed that the carnitine concentration required to support long chain fatty acid oxidation spans a wide range among different tissues of the same species (rat) and in the same tissue (skeletal muscle) across species lines (rat, dog and man). Moreover, there appeared to be a rough proportionality between the concentration needed for half-maximal rates of fatty acid oxidation and the level of this substrate normally present in tissues. To illustrate, the total carnitine content of rat liver, rat skeletal muscle and human skeletal muscle is about 0.12, 0.5 and 3.0 μmol/g wet wt., respectively; the concentration required for half-maximal rates of oleate oxidation in homogenates was found to be 10–15 μM, 40–50 μM and 200–400 μM, respectively (Long *et al.*, 1982).

These findings suggested that carnitine palmitoyltransferase I (CPT I; EC 2.3.1.21), the enzyme catalysing the first step specific to mitochondrial long chain fatty acid oxidation, exhibits vastly different requirements for carnitine depending upon the tissue in question. The present studies were designed to test this possibility by measuring directly the response of CPT I to carnitine in mitochondria isolated from various tissues of experimental animals and man. In parallel experiments we examined the sensitivity of the enzyme to inhibition by malonyl-CoA since we (J. D. McGarry, S. E. Mills, C. S. Long & D. W. Foster, unpublished work) and others (Saggerson & Carpenter, 1981) have observed that this also varies markedly in different tissues. The results show that tissues exhibiting the greatest sensitivity of CPT I to malonyl-CoA are those requiring the highest concentration of carnitine to

Abbreviation used: CPT, carnitine palmitoyltransferase.

* To whom correspondence and reprint requests should be addressed.

drive the reaction. It is also demonstrated that rat heart and skeletal muscle contain significant quantities of malonyl-CoA and that, as in the case of liver, these are decreased with starvation. Finally, it is shown that CPT I in human liver is as sensitive to inhibition by malonyl-CoA as the enzyme in experimental animals, suggesting that the scheme for control of fatty acid oxidation and ketogenesis developed from studies in rats (McGarry & Foster, 1980) is also applicable in man.

Experimental

Tissues studied

Unless otherwise stated, liver, heart and quadriceps muscle were taken from fed, male Sprague–Dawley rats (approx. 150g) that had been maintained on a high-sucrose, low-fat diet as described previously (McGarry *et al.*, 1978*c*). Guinea pig livers were from female animals weighing approx. 300g. Quadriceps muscle and heart tissue was taken from overnight fasted dogs, and human semitendinous muscle was obtained at surgery. Foetuses (16–18-week-old) were the source of human liver (cooled and used for experiments within 2h of death).

Preparation of mitochondria

All tissues were initially homogenized in 9vol. of cold 0.25M-sucrose using a glass Dounce homogenizer (liver) or a Tekmar Company Ultra-Turrax (other tissues). For most experiments the homogenates were then processed by the method of Johnson & Lardy (1967) as described earlier (McGarry *et al.*, 1978*a*) and the final mitochondria-enriched pellet was suspended in 0.15M-KCl. Protein content was estimated by the method of Lowry *et al.* (1951). This was the standard procedure for isolation of mitochondria and will be referred to as method A.

It is recognized that mitochondria prepared as described above will be contaminated to a variable extent with other cellular organelles, most notably peroxisomes and lysosomes (Leighton *et al.*, 1968). Although these do not contain significant quantities of CPT (Markwell *et al.*, 1976), the possibility existed that such contaminants might interfere in some non-specific manner with CPT I assays in mitochondria. Accordingly, in the case of rat liver and skeletal muscle two additional procedures were employed for the isolation of mitochondria. In method B the final suspension obtained from method A was concentrated five-fold in 0.15M-KCl and 1ml aliquots were layered over 10ml of 50% (v/v) Percoll/0.15M-KCl in 15ml glass tubes. The tubes were then centrifuged at 17000*g* for 2h in the SS-34 angle-head rotor of a refrigerated Sorvall centrifuge. Mitochondria were recovered in a tight band of 2ml or less at densities of 1.05–1.06 (liver) and 1.03–1.05 (muscle) as determined by density marker beads. In method C mitochondria were isolated from the low-speed nuclear pellet of the original homogenate (rather than from the low-speed supernatant as in method A) by a modification of the procedure of Fleischer & Kervina (1974). In this case tissue homogenates were centrifuged at 1000*g* for 15min, the supernatant was discarded and replaced with an equivalent volume of 0.25M-sucrose. The pellet was rehomogenized and centrifuged at 600*g* for 10min. Further centrifugation of the resulting supernatant at 15000*g* for 15min yielded a pellet rich in mitochondria. After successive washes (and recentrifugation at 15000*g* for 15min) in 0.25M-sucrose and 0.15M-KCl the final pellet was resuspended in the latter.

Mitochondrial suspensions prepared by methods A, B and C were treated with sodium taurocholate (1% final concn.) and assayed by conventional spectrophotometric techniques for marker enzymes of mitochondria, peroxisomes and lysosomes (glutamate dehydrogenase and citrate synthase for mitochondria, catalase and urate oxidase for peroxisomes, and acid phosphatase for lysosomes). Muscle mitochondria prepared by all three methods were rich in mitochondrial enzymes and showed negligible peroxisomal or lysosomal contamination. On the other hand, liver mitochondria prepared by method A contained significant quantities of peroxisomal and lysosomal enzymes. These were reduced by some 30% in preparations using method B and by 90–95% when method C was employed.

Assay of CPT I

A modification of 'assay II', described in McGarry *et al.* (1978*a*), which measures the rate of formation of palmitoylcarnitine from palmitoyl-CoA plus carnitine, was employed. Reactions were carried out at 30°C in glass culture tubes (13mm × 100mm). The standard incubation mixture initially contained, in a volume of 0.9 ml: Tris/HCl (pH 7.4), 105μmol; reduced glutathione, 0.25μmol; ATP, 4μmol, $MgCl_2$, 4μmol; KCl, 15μmol; KCN, 2μmol; rotenone, 40μg; defatted albumin, 10mg; palmitoyl-CoA, 50nmol; L-carnitine, 200nmol; and DL-[*methyl*-^{14}C]carnitine, 1μCi. Reactions were initiated by the addition of 0.1ml of the mitochondria/KCl suspension (0.2–0.5mg of protein) and were terminated with 1ml of 1.2M-HCl. The labelled palmitoylcarnitine formed was extracted and counted as described earlier (McGarry *et al.*, 1978*a*). When used, malonyl-CoA was included in this standard incubation mixture at the concentrations indicated in the Figures. In experiments where the concentration of carnitine was varied, its specific radioactivity was kept constant.

For the present studies three potential problems existed. The first was the tendency of reaction rates to decelerate with time due to the combined effects

of mitochondrial palmitoyl-CoA deacylase and the occurrence of the reverse reaction (palmitoylcarnitine + CoASH → palmitoyl-CoA + carnitine). Near linear rates were achieved by keeping incubation times short (maximum 8 min) and including ATP in the assay to allow the re-formation of palmitoyl-CoA from the free palmitic acid and CoASH generated in the deacylase reaction. [Linearity of the assay could also be improved by the addition of sodium tetrathionate to trap the free CoASH liberated. However, this agent was not employed because it was found to reduce the sensitivity of CPT I to malonyl-CoA, an observation consistent with the effects of other thiol reagents as reported by Saggerson & Carpenter (1982).] Second, it was essential that the product of the reaction, palmitoylcarnitine, not be further metabolized. This was verified in separate experiments which showed that the oxidation of [1-^{14}C]palmitoylcarnitine to acid-soluble products was effectively blocked by the presence of KCN and rotenone. Finally, it was necessary to establish that we were measuring the activity of CPT I (present on the outer aspect of the mitochondrial inner membrane) in the absence of a significant contribution from CPT II (thought to be located within the inner membrane). This was tested by evaluating suppression of activity at high malonyl-CoA concentrations [only CPT I is sensitive to the CoA ester (McGarry *et al.*, 1978*a*)]. With most mitochondrial preparations the formation of palmitoylcarnitine could be almost completely suppressed by malonyl-CoA, suggesting that CPT II activity was small or absent. In the case of dog heart and skeletal muscle, however, approx. 20% of enzyme activity appeared to be resistant to malonyl-CoA inhibition. We suspect that the non-suppressible fraction represented CPT II activity expressed as a result of damage to the mitochondria during their preparation. This follows from the observation that, when mitochondria were purposely disrupted by freeze–thawing, total CPT activity invariably increased with the malonyl-CoA-insensitive fraction generally rising to the region of 50% (see also McGarry *et al.*, 1978*a*; McGarry & Foster, 1981). To obtain a more accurate estimate of the malonyl-CoA sensitivity of CPT I in these tissues the contribution of non-suppressible enzyme was calculated from plots of (% inhibition)$^{-1}$ versus [malonyl-CoA]$^{-1}$. This quantity was then subtracted from the total activity measured in the absence and presence of the inhibitor, and the corrected values were used to construct graphs such as those shown in Fig. 1(*b*).

Analytical procedures

Liver, heart and quadriceps muscle from anaesthetized animals were rapidly frozen in liquid N_2 and extracted with perchloric acid. The neutralized extracts were analysed for malonyl-CoA content as described by McGarry *et al.* (1978*c*). Tissue levels of carnitine (free plus esterified) were measured as described by Long *et al.* (1982).

Materials

Rotenone was from Sigma. The sources of other materials have been given previously (McGarry *et al.*, 1978*a*).

Results

The response of CPT I to increasing concentrations of carnitine and the sensitivity of the enzyme to malonyl-CoA was studied in mitochondria from eight tissues prepared by method A. To simplify presentation only three representative curves are shown in Fig. 1, but the kinetic constants for all tissues are displayed in Table 1. The K_m for carnitine was found to span a range of more than 20-fold, with rat liver and dog heart showing the lowest (32 μM) and highest (695 μM) values, respectively. Because some tissues had calculated K_m values greater than the highest carnitine level routinely employed in the assay (400 μM) additional experiments were carried out using a range of carnitine concentrations from 25 μM to 3.0 mM. Under these conditions results similar to those given in Table 1 were obtained (Fig. 2).

At a fixed concentration of carnitine (200 μM), CPT I of rat liver and human foetal liver mitochondria, which exhibited equally low K_m values for carnitine, showed similar sensitivities to malonyl-CoA. The I_{50} value of 2.7 μM found with rat liver was similar to that (1.5 μM) previously reported from this laboratory (McGarry *et al.*, 1978*a*; McGarry & Foster, 1981); the small difference probably stems from the higher [palmitoyl-CoA]:[albumin] ratio employed in the present experiments since malonyl-CoA is competitive with long chain acyl-CoA towards CPT I (McGarry *et al.*, 1978*a*). Of particular interest was the finding that, as the K_m for carnitine increased, the I_{50} for malonyl-CoA fell sharply. Thus, rat heart, which had a K_m for carnitine five times higher than that of human foetal liver, required an order of magnitude less malonyl-CoA for 50% suppression of enzyme activity. This trend continued such that tissues with K_m values for carnitine in the 600–700 μM range (dog skeletal muscle and heart) had I_{50} values for malonyl-CoA of only 20 nM, i.e., they exhibited a sensitivity to the inhibitor some 100 times greater than that of rat liver.

Interestingly, as noted in the legend to Table 1, computer analysis of the data revealed a linear relationship between log (I_{50} for malonyl-CoA) and

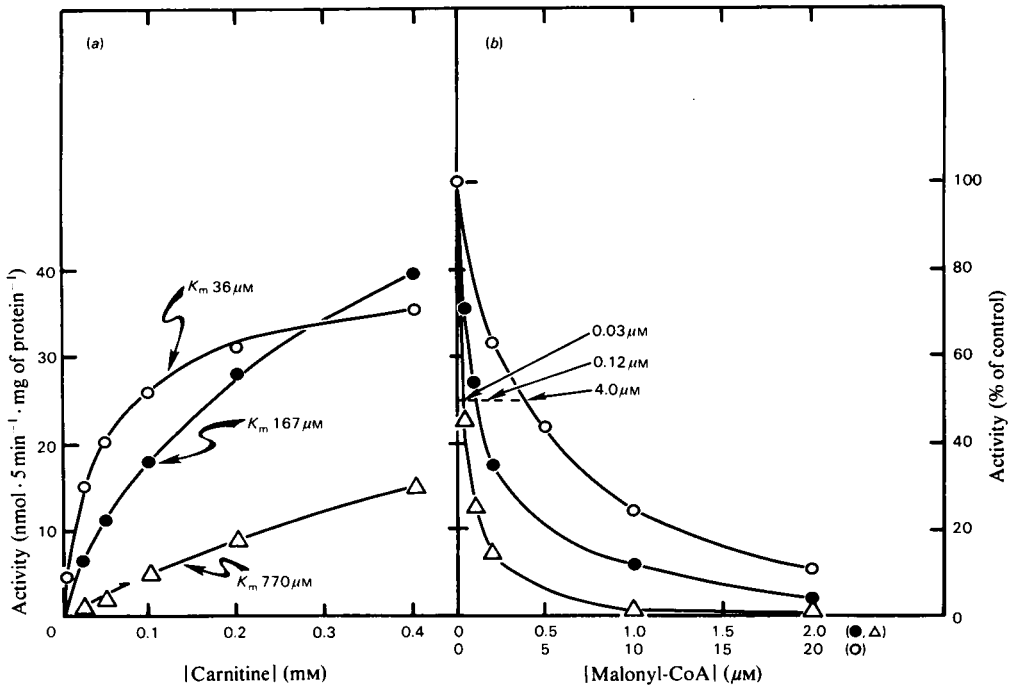

Fig. 1. *Response of mitochondrial CPT I to carnitine and malonyl-CoA*

Enzyme activity was measured in mitochondria prepared by method A as described in the Experimental section. The tissues studied were: ○, rat liver; ●, rat heart; △, dog heart. In (*b*) the concentrations of malonyl-CoA required for 50% inhibition of CPT I are indicated (note different scales on the abscissa). Values are averages of two experiments in each case.

Table 1. *Carnitine kinetics and malonyl-CoA sensitivity of mitochondrial CPT I from various tissues*

Values for the K_m for carnitine were obtained from Lineweaver–Burk plots of data such as those shown in Fig. 1(*a*) and Fig. 2. Malonyl-CoA sensitivity was calculated from plots such as those shown in Fig. 1(*b*) after correction for malonyl-CoA-insensitive activity (see the Experimental section). The term I_{50} refers to the concentration of malonyl-CoA required for 50% inhibition of enzyme activity. Data are presented as means ± S.E.M. for the number of observations shown in parentheses. The relationship between log(I_{50} for malonyl-CoA) (y) and log(K_m for carnitine) (x) is described by a linear equation: $y = -0.157x + 2.76$ ($r = 0.98$; $P < 0.0001$).

Tissue	K_m for carnitine (μM)	I_{50} for malonyl-CoA (μM)
Rat liver	32 ± 1.4 (6)	2.7 ± 0.4 (8)
Human foetal liver	39 (2)	1.6 (2)
Rat heart	197 ± 25 (4)	0.10 ± 0.01 (12)
Guinea pig liver	311 ± 32 (3)	0.16 ± 0.06 (3)
Human skeletal muscle	480 (1)	0.025 (1)
Rat skeletal muscle	507 ± 56 (5)	0.034 ± 0.006 (9)
Dog skeletal muscle	627 ± 90 (4)	0.017 ± 0.004 (4)
Dog heart	695 ± 88 (4)	0.025 ± 0.009 (4)

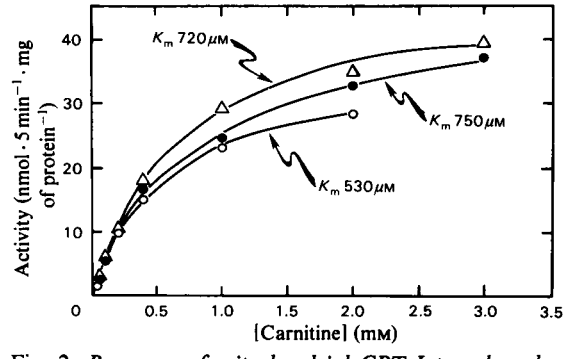

Fig. 2. *Response of mitochondrial CPT I to a broader range of carnitine concentrations*

Enzyme activity was measured as described for Fig. 1 using mitochondria from dog heart (△), dog skeletal muscle (●) and rat skeletal muscle (○). Each curve represents the average of two experiments.

log(K_m for carnitine). However, the physiological significance of this correlation, if any, is unclear.

In order to show that the differences in CPT I from various tissues were intrinsic to the enzyme and

not the consequence of modulations in the extracts, liver and muscle were combined prior to the initial homogenization step. The mixed population of mitochondria obtained displayed a distinctly biphasic response to malonyl-CoA that was entirely in keeping with the I_{50} values seen when the two tissues were processed separately, suggesting that inhibitors or activators of CPT I did not play a significant role (results not shown). In the case of rat liver and skeletal muscle all of the experiments described above were repeated using mitochondria prepared by methods B and C (see the Experimental section) in which contamination by peroxisomes and lysosomes was either reduced or eliminated. The results obtained were essentially identical with those presented in Fig. 1 and Table 1 (results not shown). It thus seems reasonable to conclude that the kinetic characteristics of CPT I in the various tissues represent genuine features of the mitochondrial enzyme.

Using mitochondria from rat liver and skeletal muscle to represent extremes of the K_m for carnitine and the I_{50} for the malonyl-CoA the following additional points were established. Increasing the concentration of carnitine from 200 to 600 μM gave no relief of the malonyl-CoA inhibition of CPT I, a finding previously reported for mitochondria from rat liver (McGarry *et al.*, 1977) and heart (Saggerson, 1982). At standard concentrations of carnitine and albumin (200 μM and 1%, respectively) the response of both types of mitochondria to increasing concentrations of palmitoyl-CoA in the presence and absence of malonyl-CoA was qualitatively similar (Figs. 3 and 4). From such experiments it was evident that the apparent K_m for palmitoyl-CoA was not greatly different in mitochondria from liver and skeletal muscle, the value ranging from 30 to 60 μM in both systems. As noted previously (McGarry *et al.*, 1978*a*; Bremer, 1981; Saggerson, 1982), accurate K_i values for malonyl-CoA could not be

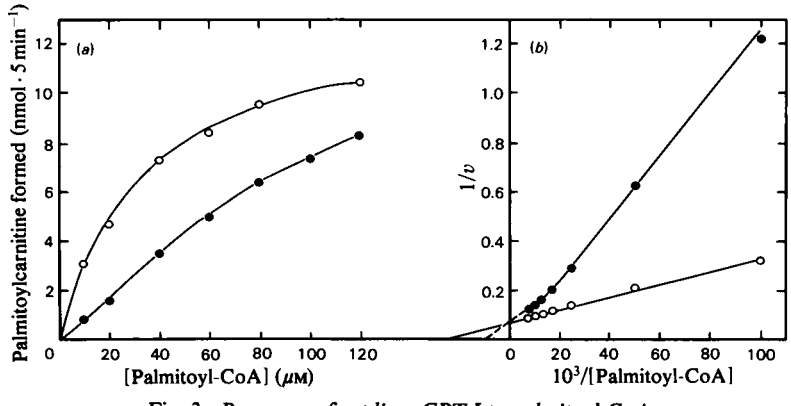

Fig. 3. *Response of rat liver CPT I to palmitoyl-CoA*

Enzyme activity was measured in liver mitochondria prepared by method A as described in the Experimental section. Representative data from three closely agreeing experiments are shown. ○, Control; ●, + 5 μM-malonyl-CoA.

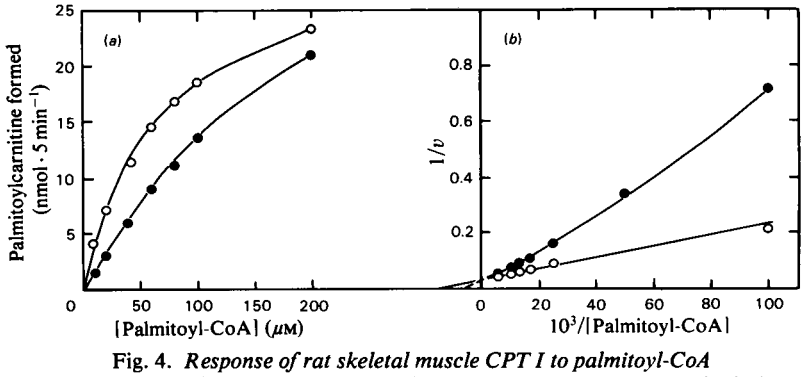

Fig. 4. *Response of rat skeletal muscle CPT I to palmitoyl-CoA*

Enzyme activity was measured in skeletal muscle mitochondria prepared by method A as described in the Experimental section. Representative data from three closely agreeing experiments are shown. ○, Control; ●, +0.05 μM-malonyl-CoA.

Table 2. *Malonyl-CoA content of liver, heart and skeletal muscle from fed and fasted rats*
Values are means ± S.E.M. for the number of measurements shown in parentheses.

State of animal	Tissue ...	Malonyl-CoA content (nmol/g wet wt.) Liver	Heart	Skeletal muscle
Fed		7.5 ± 1.2 (5)	4.6 ± 0.49 (5)	2.1 ± 0.22 (5)
Starved for 24 h		1.7 ± 0.45 (4)	1.4 ± 0.18 (5)	0.7 ± 0.09 (5)
Starved for 48 h		1.7 ± 0.87 (3)	1.3 ± 0.4 (2)	0.4 ± 0.12 (3)

obtained because of the sigmoidicity introduced into the velocity versus [palmitoyl-CoA] curve by malonyl-CoA (Figs. 3*a* and 4*a*) which resulted in non-linearity of the reciprocal plots when the inhibitor was present (Figs. 3*b* and 4*b*). Nevertheless, the fact that inhibition by malonyl-CoA was much stronger at lower than at higher concentrations of palmitoyl-CoA supports our earlier conclusion that the two CoA esters interact in 'competitive' fashion when CPT I is present on the mitochondrial membrane.

Finally, the exquisite sensitivity to malonyl-CoA of CPT I from rat heart and skeletal muscle prompted us to examine these tissues for the presence of the CoA ester. As seen from Table 2, in the fed state both heart and skeletal muscle contained readily measurable levels of malonyl-CoA (4.6 and 2.1 nmol/g wet wt., respectively), the equivalent value for liver being 7.5 nmol/g wet wt. As expected, the malonyl-CoA content of liver was much lower in starved animals. Interestingly, the same pattern was seen in heart and skeletal muscle. In all cases prior treatment of the tissue extracts with highly purified malonyl-CoA decarboxylase (kindly supplied by Dr. P. E. Kolattukudy, Washington State University) resulted in a complete loss of response in the assay system (results not shown), indicating that the compound measured in untreated extracts was indeed malonyl-CoA.

Discussion

We previously suggested that the markedly different concentrations of carnitine needed to support long chain fatty acid oxidation in crude homogenates of various mammalian tissues are likely to reflect differences in the requirement of CPT I for this substrate (Long *et al.*, 1982). The present studies, in which CPT I itself has been assayed in isolated mitochondria, provide direct proof for this concept. The data presented establish that the K_m of this enzyme for carnitine is not fixed, but, as in the case of carnitine content, varies greatly depending upon the tissue and species in question. It should be noted that for any given tissue the K_m of CPT I for carnitine (the present paper) was considerably higher than the concentration previously found necessary for half-maximal rates of overall fatty acid oxidation (Long *et al.*, 1982). The explanation lies in the fact that, as the concentration of carnitine is increased, a stage is reached at which a step distal to CPT I, probably within the β-oxidation sequence, becomes limiting for overall fatty acid oxidation (Long *et al.*, 1982; McGarry *et al.*, 1978*b*). Stated in another way, at a fixed concentration of acyl-CoA titration with carnitine will yield a maximal rate of carbon flow through the β-oxidation pathway at a time when the generation of acylcarnitine is still increasing. This point takes on added significance when the relationship between the K_m for carnitine and the total carnitine content of tissues is considered. While the two parameters generally shifted in parallel (Long *et al.*, 1982, and the present paper) the latter was found to be markedly greater than the former, suggesting that the activity of CPT I is not limited by the availability of carnitine under normal circumstances.

In contrast to the situation with carnitine the requirement of CPT I for palmitoyl-CoA does not appear to vary greatly among tissues. Under our conditions of assay apparent K_m values for this substrate were in the region of 30–60 μM for both rat liver and skeletal muscle, tissues that displayed a 20-fold difference in their K_m for carnitine. A similar value for rat heart could be deduced from the studies of Saggerson (1982).

The ability of malonyl-CoA to inhibit CPT I, a phenomenon first observed in rat liver (McGarry *et al.*, 1977) and subsequently in non-hepatic tissues of the rat (McGarry *et al.*, 1978*b*; Saggerson & Carpenter, 1981), is now seen to apply to other mammalian species including man. While the inhibitory potency of malonyl-CoA towards CPT I was of similar magnitude in liver mitochondria from the adult rat and human foetus it was one to two orders of magnitude greater in the other tissues examined. As regards rat liver, heart and skeletal muscle our findings are in essential agreement with those of Saggerson & Carpenter (1981). Although more tissues must be studied to establish the point absolutely, the present series suggests that an inverse relationship exists between the apparent K_m for carnitine and the concentration of malonyl-CoA needed for effective inhibition of CPT I. Such a

result might have been expected if malonyl-CoA were competitive with carnitine in the CPT I reaction. However, as noted above, this is not the case; malonyl-CoA is competitive towards palmitoyl-CoA.

A physiological basis for the malonyl-CoA inhibition of CPT I is well-established for rat liver, where the CoA ester is believed to play a central role in the reciprocal control of fatty acid synthesis and oxidation and in the regulation of ketogenesis (McGarry & Foster, 1980). The present findings strongly suggest that the same system operates in human liver. Surprisingly, tissues not thought to possess an active *de novo* lipogenic pathway [e.g. guinea pig liver (Patel & Hanson, 1974), skeletal muscle, heart] were found to be most sensitive to malonyl-CoA inhibition. The same point was made by Saggerson & Carpenter (1981). To our knowledge values for the malonyl-CoA content of tissues other than liver have not previously been reported, although through personal communication we understand that Dr. J. R. Neely (Milton S. Hershey Medical Center, Hershey, PA, U.S.A.) has detected it in rat heart. When we assayed rat heart and skeletal muscle significant quantities were also found. Moreover, as in the case of liver, levels were found to fall during starvation. Specificity of the assay was assured through use of a highly purified malonyl-CoA decarboxylase. A major problem in the interpretation of these observations is that we have measured only the total tissue content of malonyl-CoA. It seems reasonable to assume that in liver, which contains high levels of acetyl-CoA carboxylase in the extramitochondrial compartment, the bulk of the malonyl-CoA is present extramitochondrially and therefore available to interact with CPT I. However, the distribution of malonyl-CoA in heart and skeletal muscle (which are believed to contain little if any acetyl-CoA carboxylase) is not as certain. The intriguing feature of the data of Table 2 is that if as little as 10% of the malonyl-CoA in heart and skeletal muscle were present in the extramitochondrial space, significant inhibition of CPT I might be expected, particularly in fed animals.

A number of questions are raised by these results. For example, is CPT I in different tissues of a given species the same enzyme protein or does it exist as a variety of isoenzymes? Does the positioning of the enzyme on the mitochondrial inner membrane vary from tissue to tissue? If so, might this account for the wide range in affinity for carnitine and, *pari passu*, the dramatic shifts in sensitivity to malonyl-CoA? Does such positioning account for loss of malonyl-CoA sensitivity when the enzyme is solubilized (McGarry *et al.*, 1978*a*)? Is it possible that under certain circumstances malonyl-CoA can be formed in the extramitochondrial compartment of non-lipogenic tissues and thus play a role in the regulation of fatty acid oxidation? Theoretically, this could occur either through the action of vestigial quantities of acetyl-CoA carboxylase or, alternatively, through the intramitochondrial conversion of acetyl-CoA into malonyl-CoA via propionyl-CoA carboxylase, followed by a carnitine-linked transfer of the malonyl group to extramitochondrial CoASH. It is conceivable, of course, that the interaction between malonyl-CoA and CPT I seen in isolated mitochondria from non-lipogenic organs is purely fortuitous in physiological terms and that in intact cells of these tissues another metabolite, perhaps chemically related to malonyl-CoA, serves as a regulator of fatty acid oxidation. Answers to these questions are currently being sought.

In the meantime it appears safe, on the basis of the present results, to extend the domain of malonyl-CoA-controlled lipid metabolism to human liver and attractive to speculate that the regulatory role of this simple molecule might be broader than previously suspected.

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