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Time-dependence of inhibition of carnitine palmitoyltransferase I by malonyl-CoA in mitochondria isolated from livers of fed or starved rats

Evidence for transition of the enzyme between states of low and high affinity for malonyl-CoA

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1. The degree of inhibition of CPT I (carnitine palmitoyltransferase, EC 2.3.1.21) in isolated rat liver mitochondria by malonyl-CoA was studied by measuring the activity of the enzyme over a short period (15s) after exposure of the mitochondria to malonyl-CoA for different lengths of time. 2. Inhibition of CPT I by malonyl-CoA was markedly time-dependent, and the increase occurred at the same rate in the presence or absence of palmitoyl-CoA (80 μM), and in the presence of carnitine, such that the time-course of acylcarnitine formation deviated markedly from linearity when CPT I activity was measured in the presence of malonyl-CoA over several minutes. 3. The initial rate of increase in degree of inhibition with time was independent of malonyl-CoA concentration. 4. CPT I in mitochondria from 48h-starved rats had a lower degree of inhibition by malonyl-CoA at zero time, but was equally capable of being sensitized to malonyl-CoA, as judged by an initial rate of increase of inhibition identical with that of the enzyme in mitochondria from fed rats. 5. Double-reciprocal plots for the degree of inhibition produced by different malonyl-CoA concentrations at zero time for the enzyme in mitochondria from fed or starved animals indicated that the enzyme in the latter mitochondria was predominantly in a state with low affinity for malonyl-CoA (concentration required to give 50% inhibition, $I_{0.5} \simeq 10\,\mu$M), whereas that in mitochondria from fed rats displayed two distinct sets of affinities: low ($\simeq 10\,\mu$M) and high ($<0.3\,\mu$M). Plots for mitochondria after incubation for 0.5 or 1 min with malonyl-CoA indicated that the increased sensitivity observed with time was due to a gradual increase in the high-affinity state in both types of mitochondria. 6. These results suggest that the sensitivity of CPT I in rat liver mitochondria *in vitro* had two components: (i) an instantaneous sensitivity inherent to the enzyme which depends on the nutritional state of the animal from which the mitochondria are isolated, and (ii) a slow, malonyl-CoA-induced, time-dependent increase in sensitivity. 7. It is suggested that the rate of malonyl-CoA-induced sensitization of the enzyme to malonyl-CoA inhibition is limited by a slow first-order process, which occurs after the primary event of interaction of malonyl-CoA with the mitochondria. 8. The putative role of such a process in a possible mechanism whereby differences in sensitivity *in vivo* (related to differences in hepatic malonyl-CoA concentrations) may be partially retained *in vitro* is discussed.

The sensitivity of overt CPT activity in isolated rat liver mitochondria to malonyl-CoA inhibition is higher in mitochondria isolated from animals in physiological states characterized by low rates of hepatic fatty acid oxidation and high concentrations of malonyl-CoA in the liver (Saggerson & Carpenter, 1981; Robinson & Zammit, 1982; Stakkestad & Bremer, 1983). Two observations have suggested that this increased sensitivity could be mediated, at least in part, by a malonyl-CoA-induced sensitization of the enzyme *in vivo*. Firstly,

Abbreviation used: CPT I, carnitine palmitoyltransferase I (EC 2.3.1.21).

the sensitivity of the enzyme in isolated mitochondria was found to be directly and quantitatively related to the hepatic concentration of malonyl-CoA *in vivo* (Robinson & Zammit, 1982), and secondly, the increased sensitivity could be mimicked *in vitro* by preincubation of mitochondria with physiological concentrations of malonyl-CoA (Zammit, 1983). The physiological significance of malonyl-CoA-induced malonyl-CoA inhibition of the CPT I would reside in the potent amplification of the effect that relatively small changes in hepatic malonyl-CoA concentration would have on the activity of the enzyme, and thence on the rate of fatty oxidation, were such a mechanism to operate *in vivo* (Robinson & Zammit, 1982).

The malonyl-CoA-induced sensitization of CPT I *in vitro* in liver mitochondria was previously studied under conditions that did not allow the time-dependence of the phenomenon to be investigated, since preincubations were performed at 0°C and in dilute mitochondrial suspension to minimize breakdown of malonyl-CoA by the mitochondria (Zammit, 1983). However, if the conclusions of that study are valid, it should be possible to follow the progress of malonyl-CoA-induced sensitization by measuring the activity of the enzyme over very short assay times in mitochondria incubated with malonyl-CoA for different periods of time at 37°C before initiation of the enzyme assay by addition of substrates. If sensitization does occur, the degree of inhibition caused by any one chosen concentration of malonyl-CoA should increase in a time-dependent manner. A study of the characteristics of the time-course of such a phenomenon should yield useful information about the mechanism(s) involved.

The present paper reports the results of such experiments. A markedly time-dependent increase in the degree of inhibition of CPT I by physiological concentrations of malonyl-CoA was observed. The initial rate of this sensitization was independent of malonyl-CoA concentration and of the presence or absence of palmitoyl-CoA and was identical for the enzyme in mitochondria isolated from fed or starved rats. However, the inherent sensitivity of the enzyme, i.e. the degree of inhibition by malonyl-CoA added at the time of initiation of the assay (zero time), was strongly dependent on malonyl-CoA concentration and on the nutritional state of the animals. Evidence for the existence of two states of CPT I with a 40-fold difference in affinity for malonyl-CoA was also obtained. The significance of these observations for the regulation of CPT I activity by changes in malonyl-CoA concentration *in vivo* and the implications for the concept of sensitivity of the enzyme towards its effector *in vitro* are discussed.

Materials and methods

Animals

The source of the female virgin Wistar rats (160–200g) and their maintenance were as described previously (Zammit, 1980). Starved animals had their food removed 48h before being used, but had continuous access to water.

Chemicals

The sources of chemicals and L-[*methyl*-^{14}C]-carnitine were as described by Robinson & Zammit (1982) and Zammit (1983). In addition, Antimycin A and rotenone were from Sigma (London) Chemical Co., Poole, Dorset BH17 7NH, U.K.

Preparation of mitochondria

Mitochondria were prepared as described previously (Robinson & Zammit, 1982). They were washed once and finally resuspended in extraction medium (0.25M-sucrose, 5mM-Tris/HCl, 1mM-EGTA, pH7.4 at 0°C). The protein concentration in the final suspension was approx. 20mg/ml.

Time-course of malonyl-CoA inhibition and assay of enzyme activity

The time-course of malonyl-CoA inhibition of CPT I activity was determined by incubating mitochondria in the assay medium with a chosen concentration of malonyl-CoA for different periods of time before starting the reaction by addition of substrate(s). To keep the period of incubation of the mitochondria constant, irrespective of the length of time for which they were exposed to malonyl-CoA, and to eliminate the possibility that the sensitivity of the enzyme could be affected by incubation conditions other than the addition of malonyl-CoA, the total period of incubation before assay was always the same (7min); malonyl-CoA (10μl) was added at intervals before the start of the reaction. Thus the only factor that was varied was the length of time that elapsed between addition of malonyl-CoA and initiation of the enzyme assay. In experiments in which time-dependence of sensitization was studied in the absence of palmitoyl-CoA, the reaction was started by addition of an appropriate mixture of prewarmed solutions of palmitoyl-CoA and carnitine. In experiments in which palmitoyl-CoA was present during preincubation with malonyl-CoA, the reaction was started by the addition of carnitine only. In all instances the 'zero-time' effect of malonyl-CoA was obtained by addition of the appropriate amount of malonyl-CoA together either with palmitoyl-CoA plus carnitine or with carnitine alone at the start of the assay.

To ensure that near-instantaneous measurements of initial enzyme activity were made, the enzyme activity was measured over the shortest possible reproducible time interval (15s) which gave an easily measurable activity. This assay time also minimized any progress of sensitization during the assay itself. The final assay medium contained (in 2ml) 220mM-sucrose, 40mM-KCl, 5mM-Tris/HCl, 1mM-EGTA, 80μM-palmitoyl-CoA, 10mg of dialysed fatty-acid-poor albumin/ml, 0.4mM-L-[^{14}C]-carnitine (1Ci/mol), 1μg of Antimycin A/ml, 2μg of rotenone/ml, 1.5–2.0mg of mitochondrial protein and the concentrations of malonyl-CoA indicated in the legends of Figs. 1–4. The final pH was 7.4 at 37°C. The reactions were started by addition of carnitine or palmitoyl-CoA plus carnitine (see above) and stopped after 15s by addition of 0.3ml of 6M-HCl. The amount of acyl[^{14}C]carnitine formed was quantified as described previously (Robinson & Zammit, 1982). For the experiments reported in Fig. 3, the amount of mitochondrial protein was 0.4–0.5mg per assay tube, the reactions were started with either carnitine or a mixture of carnitine and malonyl-CoA, and stopped as described above after the time intervals shown.

Analytical methods

Protein and malonyl-CoA concentrations were measured as described previously (Zammit, 1983).

Expression of results

The inhibition of CPT I by malonyl-CoA is given as the activity of the enzyme in the presence of a given concentration of malonyl-CoA divided by that obtained in the absence of added malonyl-CoA (i.e. 'control' activity) and expressed as a percentage. The 'control' activity (nmol/15s per mg of mitochondrial protein at 37°C) of the enzyme is given in the legends to Figures. 'Separate determinations' in these legends refer to experiments on separate preparations of mitochondria from different rats.

Results

Time-dependent inhibition of CPT I by malonyl-CoA

The degree of inhibition of CPT I produced by any given concentration of malonyl-CoA was markedly dependent on the time for which the mitochondria (from fed rats) were exposed to the metabolite before the assay was started (Fig. 1). The initial rate of sensitization (i.e. the rate of increase in the degree of inhibition) was the same for all five concentrations of malonyl-CoA tested (range 0.35–14μM). Thus the activity of CPT I after 1min of incubation with malonyl-CoA as a percentage of the activity obtained when malonyl-CoA was added at zero time together with the substrate(s) was 81.8±3.0%, 80.6±3.1%, 78.8±2.1% and 79±1.0% for 0.7, 2.8, 7 and 14μM-malonyl-CoA respectively. The increase in sensitivity was linear with time for 1–2min, but slowed down progressively thereafter. This effect was greatest for the lower concentrations of malonyl-CoA and may have been due partly to the breakdown of the metabolite by the mitochondria (see below). (Although the initial rate of sensitization was independent of malonyl-CoA concentration, the absolute activity of CPT I, which was the parameter measured, was very dependent on concentration; see Figs. 1 and 5. Therefore loss of malonyl-CoA from the medium would result in an increase in measured CPT I activity at any given time point; hence the separate and differently shaped curves in Fig. 1.) In parallel experiments in which mitochon-

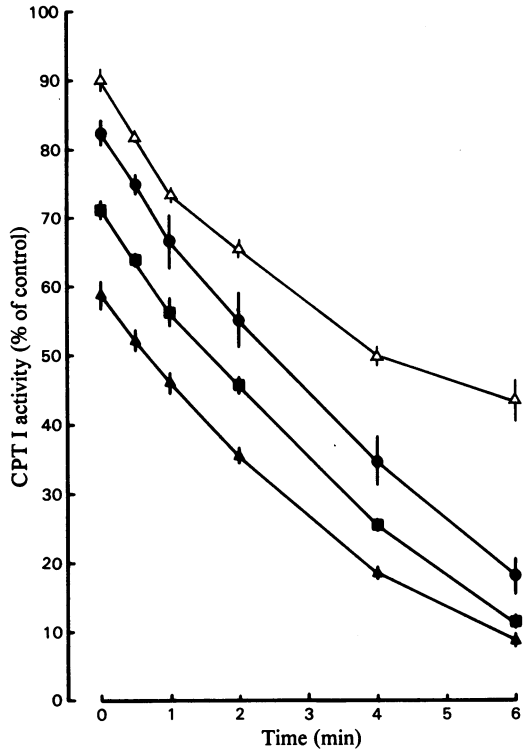

Fig. 1. *Effect of duration of incubation of isolated rat liver mitochondria with different concentrations of malonyl-CoA on the subsequently measured activity of CPT I*

Mitochondria were preincubated and CPT I activity was assayed as described in the Materials and methods section. The initial concentrations of malonyl-CoA were 0.7μM (△), 2.8μM (●), 7μM (■) and 14μM (▲). Values are means±S.E.M. for three separate determinations on different mitochondrial preparations, except for 7μM-malonyl-CoA ($n=4$). Control activities were 0.30±0.02nmol/15s per mg of mitochondrial protein. Data for 0.35μM-malonyl-CoA are omitted in the interests of clarity, but are incorporated in Figs. 5 and 6.

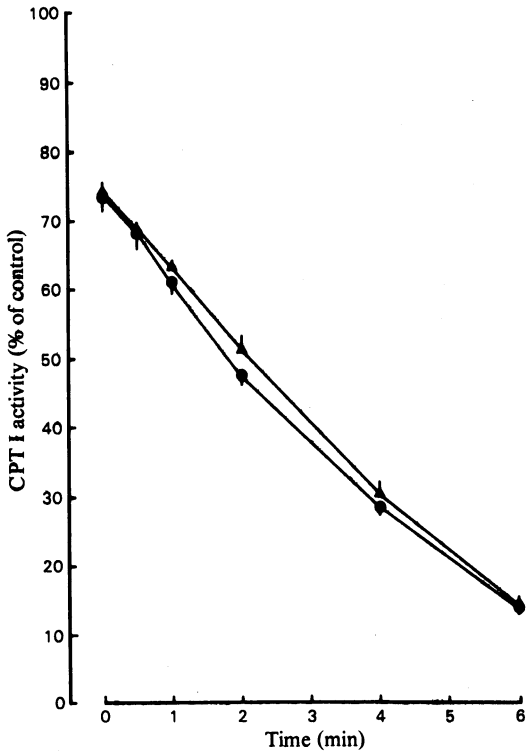

Fig. 2. *Effect of palmitoyl-CoA on the rate of increase in malonyl-CoA inhibition of CPT I*

Mitochondria were incubated with malonyl-CoA (7 μM) for the times shown in the absence (▲) or presence (●) of 80 μM-palmitoyl-CoA; assays were started by addition of carnitine/palmitoyl-CoA mixture or carnitine alone, respectively. Values are means ± S.E.M. for three separate determinations on different mitochondrial preparations. Control activities were 0.38 ± 0.04 nmol/15 s per mg of mitochondrial protein.

dria were incubated with 7 μM-malonyl-CoA initially, the malonyl-CoA concentration in the medium was decreased to between 5 and 6 μM at the end of a 6 min incubation (results not shown). Other controls, in which mitochondria were incubated for only 2 min in assay medium in the absence of any additions, confirmed that, in the absence of added malonyl-CoA, there was no difference in 'control' activity after 2 or 7 min of incubation. Therefore time-dependent increase in degree of inhibition described above was due solely to malonyl-CoA addition.

In the above experiments the enzyme assays were initiated by addition of carnitine, i.e. palmitoyl-CoA was present during the period of incubation before assay. In order to assess the possible effects of palmitoyl-CoA on the time-dependent effects of malonyl-CoA, experiments in which mitochondria were incubated with malonyl-CoA either in the presence (as described above) or in the absence of palmitoyl-CoA (80 μM) were performed. Time-courses for the increased sensitivity of the enzyme were not significantly different in the absence of palmitoyl-CoA (Fig. 2). Thus palmitoyl-CoA did not affect the rate of sensitization. Thereafter palmitoyl-CoA was included routinely during preincubation of mitochondria with malonyl-CoA.

In preliminary experiments designed to determine whether increased sensitivity could occur during the assay itself, experiments in which the assay time was prolonged to 4 min (with 0.4–0.5 mg of mitochondrial protein) were performed with several different concentrations of malonyl-CoA, and the amount of acylcarnitine formed was measured at 1 min intervals. Whereas 'control' assays from which malonyl-CoA was absent were linear for up to 4 min, assays to which malonyl-CoA was added deviated markedly from linearity and yielded an assay time-course which mirrored the time-courses of sensitization shown in Figs. 1, 2 and 4. A representative time-course is given in Fig. 3. The fact that deviation from linearity only occurred in assays in which CPT I activity was lower than in the controls excludes the possibility that it was due to depletion of palmitoyl-CoA or accumulation of products. Therefore it appears that sensitization of CPT I to malonyl-CoA inhibition occurred also during the catalysis of acylcarnitine formation and in the presence of carnitine.

Effects of starvation on zero-time sensitivity and rate of sensitization

In order to ascertain which of the two parameters which contributed towards the sensitivity of CPT I *in vitro* (i.e. sensitivity at zero-time or time-dependent increase of sensitization) was responsible for the difference in sensitivity *in vitro* reported in previous studies (see the introduction), the experiments described above were repeated with mitochondria isolated from livers of either fed rats or 48 h-starved animals. In experiments designed to study the effect of starvation on the rate of malonyl-CoA-induced sensitization, the comparison between the two types of mitochondria was made by using one chosen concentration of malonyl-CoA (7 μM). The initial rate of sensitization was identical for mitochondria from fed or starved animals (Fig. 4), although sensitization slowed down more rapidly after 2 min for 'starved' mitochondria. This may have been due to differences in the rate of malonyl-CoA breakdown. In a second series of experiments the zero-time inhibition achieved by a range of malonyl-CoA concentrations was studied (Fig. 5) for the enzyme in mitochondria from fed or starved rats. The zero-time sensitivity of CPT I activity to malonyl-CoA

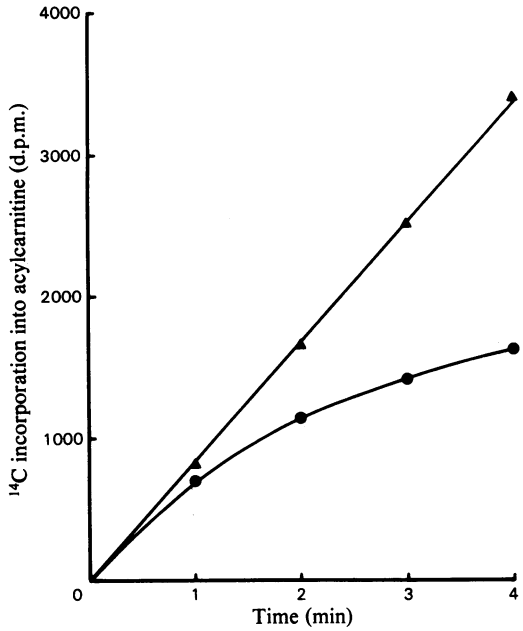

Fig. 3. *Representative time-course of ^{14}C incorporation from* L-[^{14}C]*carnitine into butanol-extractable acylcarnitine by isolated mitochondria from liver of fed rats*
Incorporation was measured at different times after the initiation of the reaction with carnitine (▲) or carnitine plus malonyl-CoA (7 μM) (●). In the experiment illustrated, the amount of mitochondrial protein in the assay medium (2 ml) was 0.44 mg and the activity in the absence of malonyl-CoA was 1.4 nmol/min per mg of mitochondrial protein. Similar time-courses were obtained for other concentrations of malonyl-CoA.

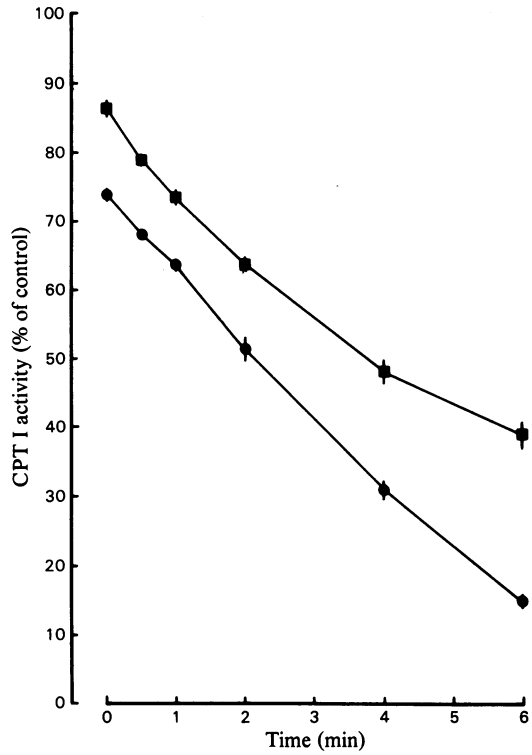

Fig. 4. *Time-dependent increase of malonyl-CoA inhibition of CPT I in mitochondria from fed or starved rats*
Mitochondria from livers of fed (●) or 48 h-starved (■) rats were incubated as described in the Materials and methods section, and CPT I activity was measured after different periods of exposure to malonyl-CoA (7 μM). Values are means ± S.E.M. for four separate determinations on different mitochondrial preparations. Control activities were 0.31 ± 0.04 and 0.46 ± 0.02 nmol/15 s per mg of protein for mitochondria from fed or starved rats respectively.

inhibition was greater in mitochondria from fed rats than in those from 48 h-starved animals. Therefore CPT I in mitochondria from animals in these two physiological conditions differed in its inherent sensitivity to malonyl-CoA, but not in the rate at which it was capable of being sensitized by malonyl-CoA. (It will be apparent that the difference in sensitivity between the two types of mitochondria was only preserved as long as the time of incubation with malonyl-CoA was kept constant. For example, Fig. 4 shows that CPT I in mitochondria from starved rats could, if these were incubated for, say, 4 min with malonyl-CoA, be made to display greater sensitivity *in vitro* than CPT I in mitochondria from fed rats, if the latter were incubated with malonyl-CoA for only 1 min.) There was, in addition, an increase in 'control' activity in mitochondria from starved rats (see legends to Figs. 4 and 5), as described previously by Saggerson & Carpenter (1982).

Double-reciprocal plots of the mean values in Fig. 5 give an insight into the basis of this inherent difference in CPT I in mitochondria from fed and starved animals. Thus the plot for fed animals has two distinct slopes (Fig. 6*a*), which yield values for the malonyl-CoA concentration required to give 50% inhibition ($I_{0.5}$) of 10 μM and about 0.25 μM, whereas CPT I in mitochondria from starved rats (Fig. 6*b*) displays primarily the lower affinity (see the legend). Moreover, the plots for CPT I after incubation of mitochondria for 0.5 and 1.0 min with malonyl-CoA (Fig. 6) indicate that the time-dependent increase in inhibition of the enzyme was due to the gradual increase in the amount of the enzyme activity in the high-affinity state at the expense of the amount of CPT I in the low-affinity state.

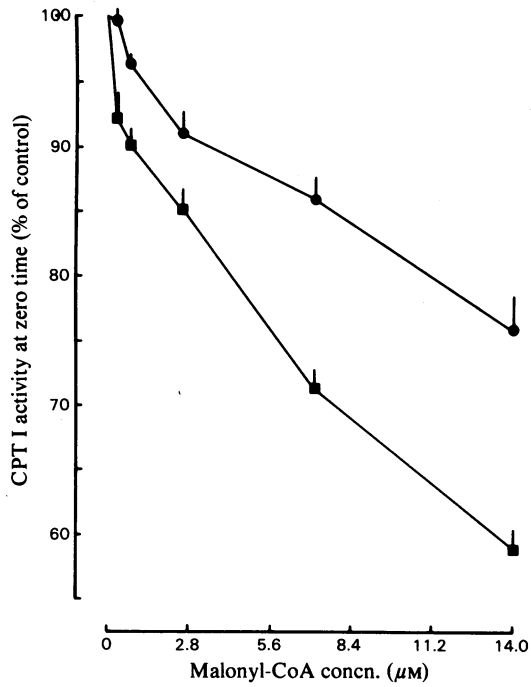

Fig. 5. *Sensitivity of CPT I in mitochondria from fed (■) or 48h-starved (●) rats to inhibition by malonyl-CoA added at zero time*

Incubations were carried out as described in the legend to Fig. 1. Assays were started by addition of mixtures of carnitine and the appropriate amounts of malonyl-CoA. Control activities for the two types of mitochondria were obtained by starting the reaction with carnitine alone. Values are means ±S.E.M. for three separate determinations on different mitochondrial preparations. Control activities were 0.31±0.04 and 0.46±0.02nmol/15s per mg of mitochondrial protein for fed and starved rats respectively.

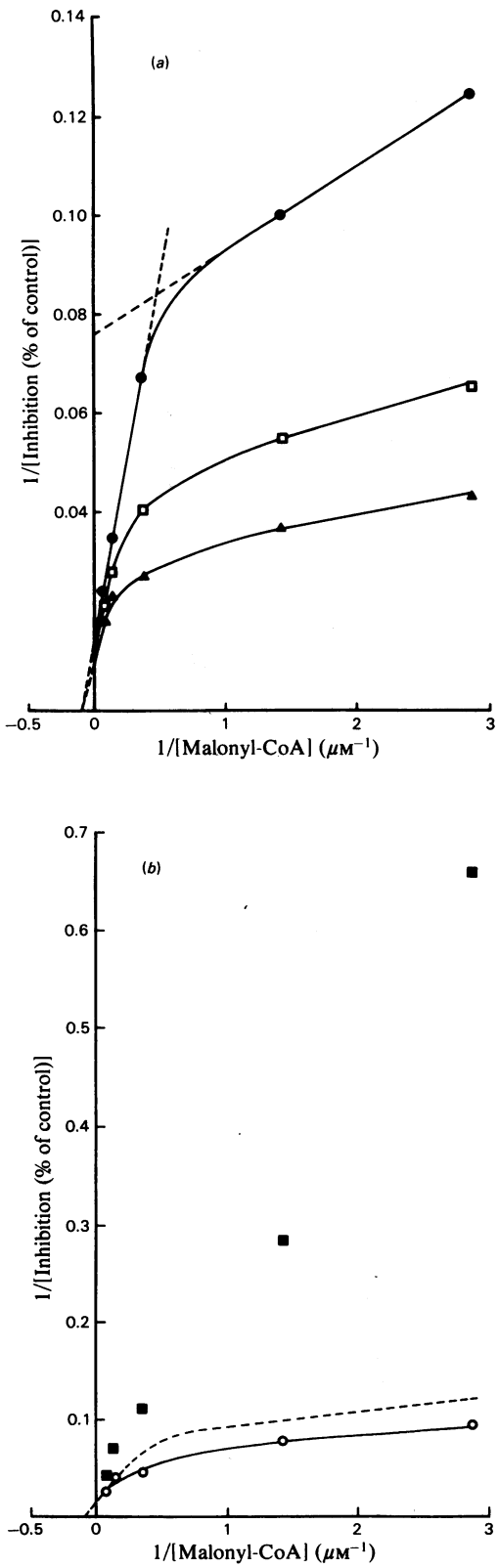

Fig. 6. *Double-reciprocal plots for degree of inhibition of CPT I activity (% of control) with increasing concentrations of malonyl-CoA*

(*a*) Mitochondria from fed rats at zero time (●) and after 0.5min (□) or 1min (▲) incubation with the respective concentrations of malonyl-CoA. (*b*) Mitochondria from starved rats at zero time (■) and after 1min of incubation (○). Data are abstracted from Figs. 1 and 5 ($n = 3$ or 4), except for (○), which are means of two separate determinations. In view of the different scales for the ordinate axes of Figs. 6(*a*) and 6(*b*), the curve for zero-time data of mitochondria from fed rats is reproduced in Fig. 6(*b*) (dashed line) to facilitate comparison between zero-time data of the two types of mitochondria. It was considered that the zero-time data for mitochondria from starved rats did not allow the unequivocal drawing of a curve through the points (■); however, the predominance of the low-affinity component ($I_{0.5} \simeq 10\,\mu M$) of the inhibition is evident.

Discussion

These results establish that sensitivity of CPT I to malonyl-CoA inhibition, as measured *in vitro*, has two distinct components: (i) an instantaneous sensitivity, which is inherent in the mitochondria and which depends on the nutritional state of the animal from which the mitochondria are obtained, and (ii) a slow, malonyl-CoA-induced, time-dependent increase in sensitivity, the initial rate of which is the same for mitochondria from fed or starved animals, and is independent of malonyl-CoA concentration or the presence of palmitoyl-CoA.

The study of component (i) shown in Fig. 5 illustrates that the inherent sensitivity of the enzyme in mitochondria as prepared is rather low, and that a higher sensitivity may be observed *in vitro*, owing to the increase in sensitivity that occurs in the assay medium itself when the mitochondria are exposed to malonyl-CoA before (Figs. 1, 2 and 4) and during (Fig. 3) assay of enzyme activity, particularly if the latter is done over a period of several minutes. Unfortunately the wide ranges of preincubation and assay times (in addition to substrate concentrations) reported in the literature make it impossible directly to compare results obtained from different laboratories in the light of the present observations. [Although CPT I in mitochondria from both fed or starved animals is inhibited in a time-dependent manner by malonyl-CoA, because of the characteristics and combination of components (i) and (ii) the relative difference in sensitivity (as defined in previous studies; see, e.g., Cook *et al.*, 1983) would be retained and indeed increased were longer assay times to be used (as in previous studies), since the relative 'activities' measured would be represented by the areas under the two curves shown in Fig. 4, provided that the total periods of exposure of the mitochondria to malonyl-CoA were always maintained constant.]

The investigation of component (ii) of the sensitivity of CPT I to malonyl-CoA inhibition *in vitro* verifies the prediction, which stemmed from earlier experiments (Zammit, 1983), that, if malonyl-CoA is capable of inducing sensitivity to CPT I, it should be possible to follow the progress of this sensitization by measuring the activity of the enzyme over short periods of time. The rate of sensitization was slow, such that the degree of inhibition of the enzyme after incubation of the mitochondria with malonyl-CoA for 1 min at 37°C under the present conditions was about 20% greater than that observed when malonyl-CoA was added at the time the assay was started. In addition, this rate was independent of malonyl-CoA concentration in the range studied (which also corresponds to the physiological range of concentrations; see Zammit, 1981). It is suggested that the rate of the observed malonyl-CoA-induced sensitization of CPT I to malonyl-CoA inhibition was limited by a slow first-order process. The fact that this process was unaffected by palmitoyl-CoA suggests that it is distinct from the well-documented mutually competitive effects of malonyl-CoA and palmitoyl-CoA.

As far as I am aware, the phenomenon most closely analogous to component (ii) that has been reported in the literature is the time-dependent increase in affinity for peptide hormones by receptors in plasma membranes incubated with the hormones *in vitro* (e.g. Donner & Corin, 1980).

Physiological considerations

The physiological relevance of the present findings is evident when they are considered in conjunction with two other sets of observations, namely that *in vivo*, under conditions of increased flux through the reaction catalysed by CPT I, the concentration of malonyl-CoA is lower in the liver (Guynn *et al.*, 1972; Cook *et al.*, 1977; Lynen, 1979; Zammit, 1981), and that the sensitivity of CPT I in isolated mitochondria is directly related to the concentration of malonyl-CoA *in vivo* (Robinson & Zammit, 1982). Thus, if the sensitization of CPT I observed *in vitro* occurs also *in vivo* and is freely reversible (as seems reasonable to assume if the enzyme is not to be permanently inhibited), it is possible that a steady-state degree of sensitivity appropriate to the concentration of malonyl-CoA obtaining in the liver *in vivo* could be achieved. The implications that such a relationship between malonyl-CoA concentration and sensitivity of CPT I to malonyl-CoA inhibition *in vivo* would have for amplification of the effects of changes in hepatic malonyl-CoA concentration on the rate of non-esterified fatty acid oxidation have been outlined previously (Robinson & Zammit, 1982; Zammit, 1983; see also the introduction).

The fact that CPT I in mitochondria from fed or starved rats was equally capable of being sensitized by malonyl-CoA suggests that the enzyme in mitochondria from rats in these two nutritional states was not fundamentally different and that in either type of mitochondria CPT I could display a wide range of inhibition by malonyl-CoA, depending on the length of exposure of the mitochondria to the metabolite. As described above, the difference in zero-time sensitivity in the two types of mitochondria could be a consequence of the fact that the hepatic concentrations of malonyl-CoA in the two nutritional conditions were different, such that the enzyme existed in different steady states of sensitivity *in vivo* (see above and Robinson & Zammit, 1982). In this respect it was particularly interesting to observe that, as indicated by the double-reciprocal plots in Fig. 6, whereas CPT I in mitochon-

dria from starved animals existed mostly in a state with low affinity for malonyl-CoA ($I_{0.5} \simeq 10\,\mu M$) the enzyme in mitochondria from fed animals existed in a combination of high- and low-affinity states, with $I_{0.5} < 0.3\,\mu M$ and $\simeq 10\,\mu M$, respectively. Moreover, for both types of mitochondria, as sensitization proceeded the relative amounts of the low- and high-affinity states changed, with the inhibition owing to the high-affinity state becoming progressively more important and that owing to the low-affinity state decreasing. Therefore the difference in the steady states suggested above could represent different relative amounts of low- and high-affinity states *in vivo* similar to those observed *in vitro*.

However, the mechanism whereby the differences that may exist *in vivo* could be reflected *in vitro* in the manner shown in Fig. 4 may not be immediately apparent. Indeed, the question arises as to why any difference at all should be observed *in vitro*, since the concentration of malonyl-CoA is infinitely diluted during preparation of the mitochondria. A possible explanation for this apparent paradox may reside in the slowness of the rate of increase in sensitivity observed in the studies reported in Figs. 1, 2 and 4, plus the observation that the rate of this sensitization was not dependent on malonyl-CoA concentration (Fig. 1). These results suggest that the process of transition from an insensitive to a sensitive state of CPT I occurred after a malonyl-CoA-dependent event (presumably represented by an interaction of malonyl-CoA with a component of the mitochondrial inner membrane) and that the slowness of this process limited the entire rate of sensitization and rendered this rate apparently independent of malonyl-CoA concentration. Consequently, if the sensitive-to-insensitive transition occurs through the reversal of the same rate-limiting process, its completion is likely to lag considerably behind the removal of malonyl-CoA from the medium in which the mitochondria are prepared, especially if it is considered that isolation of mitochondria is performed at temperatures near 0°C. Thus, depending on whether the limiting process has a temperature coefficient (Q_{10}) of about 2.5 (generally associated with enzyme-catalysed reactions) or higher [associated with some membrane-protein-mediated processes, e.g. $Q_{10} = 8.5$ for acylcarnitine translocation across the mitochondrial inner membrane (Ramsay & Tubbs, 1976)], desensitization could occur from several tens to several thousands of times slower at 0°C than the rate of sensitization observed at 37°C (Figs. 1, 2 and 4). That a loss of sensitivity (which is reversible by malonyl-CoA) occurs when the time between mitochondrial isolation and assay of CPT I is prolonged has been demonstrated previously ('I' versus 'M−' mitochondria; Zammit, 1983).

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