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Sensitivity of carnitine acyltransferase I to malonyl-CoA inhibition in isolated rat liver mitochondria is quantitatively related to hepatic malonyl-CoA concentration *in vivo*

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The sensitivity of carnitine acyltransferase I (EC 2.3.1.21) activity to malonyl-CoA inhibition in rat liver mitochondria isolated from animals in various physiological states was quantitatively proportional to the hepatic malonyl-CoA concentration *in vivo*. It is suggested that this relationship between the two parameters could result in a potent amplification mechanism for the reciprocal regulation of fatty acid synthesis and oxidation.

The overt activity of carnitine acyltransferase of rat liver mitochondria is inhibited by micromolar concentrations of malonyl-CoA (see McGarry & Foster, 1980, for review). Several studies have suggested that the enzyme in mitochondria isolated from livers of starved rats is less sensitive to malonyl-CoA inhibition than is the enzyme in mitochondria from fed animals (Cook *et al.*, 1980; Ontko & Johns, 1980; Saggerson & Carpenter, 1981*a*). The methodology used in those studies has been criticized, and the relatively high concentrations of malonyl-CoA required to inhibit the enzyme under the conditions used to demonstrate these alterations in sensitivity were taken to indicate that the observed changes were not physiologically relevant (McGarry & Foster, 1981). However, even in the studies of the last authors there was a significant difference in the sensitivity of the enzyme in mitochondria from fed and starved animals. Nevertheless it was concluded that the effects were not profound, and their physiological significance was questioned (McGarry & Foster, 1981).

From observations made previously in our laboratory, it was apparent that the difference in hepatic malonyl-CoA concentration *in vivo* between fed and starved virgin female animals was relatively small (2.1 nmol/g wet wt.) compared with the wide range of malonyl-CoA concentrations found in livers of animals in other physiological states, e.g. pregnancy, lactation (0.4–6.6 nmol/g wet wt.; Zammit, 1981). Hence it was possible that a much larger range of sensitivity of CAT I to malonyl-CoA inhibition could occur in mitochondria from livers of animals in these other physiological states and that a study of these animals could provide an opportunity to investigate the relationship between sensitivity of CAT I *in vitro* and hepatic malonyl-CoA concentrations *in vivo*.

Abbreviation used: CAT I, carnitine acyltransferase I (EC 2.3.1.21).

Materials and methods

Sources of chemicals were as described by Zammit (1980, 1981). DL-[*methyl*-^{14}C]Carnitine hydrochloride was obtained from Amersham International, Amersham, Bucks., U.K. Rats were obtained from A. Tuck and Sons (Battlesbridge, Essex, U.K.) and maintained as described previously (Zammit, 1980). Starved rats had their food removed 24 h before being killed, which was always performed between 09:00 and 10:00 h. Liver mitochondria were prepared as described by Chappell & Hansford (1972). CAT I activity was measured at 37°C essentially as described by Saggerson & Carpenter (1981*a*), except that the concentration of defatted albumin used was 6-fold higher, i.e. 10 mg/ml. The complete assay medium contained in 2.0 ml was 220 mM-sucrose, 40 mM-KCl, 10 mM-Tris/HCl, 1 mM-EGTA, 0.5 mM-dithiothreitol, 40 μM-palmitoyl-CoA, 20 mg of defatted albumin, 0.4 mM-L-carnitine (2 μCi/μmol) and malonyl-CoA (at five different concentrations). The final pH was 7.4. The reaction mixture with the omission of malonyl-CoA and carnitine was incubated in glass tubes at 37°C. A portion (0.1 ml) of mitochondrial suspension containing 0.2 mg of mitochondrial protein was added to individual tubes and thermal equilibration

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allowed to proceed for 1 min. The malonyl-CoA solution was then added and the reactions were started by addition of a prewarmed carnitine solution. The reactions were terminated after 1, 2 or 3 min by addition of 0.1 ml of 6 M-HCl. The amount of palmitoyl[^{14}C]carnitine formed was measured as described by Borrebaek (1975). Rates were linear with time for at least 3 min. Respiratory control ratios for mitochondria were measured routinely (in the same basic incubation medium as above, with 5 mM-glutamate and 5 mM-malate as respiratory substrates) and were found to be always above 4.5. Mitochondrial protein was measured by the method of Lowry *et al.* (1951), with bovine albumin as standard.

Results and discussion

The activity of CAT I in the absence of added malonyl-CoA was essentially the same (0.3 nmol/min per mg of protein) in mitochondria from livers of all the animals studied. This activity was low compared with the maximal activity of the enzyme (approx. 6 nmol/min per mg of protein; cf. data of Saggerson & Topping, 1981) and was due to the low concentration of palmitoyl-CoA used in order to ensure the functional integrity of the mitochondria and to avoid the problems outlined by McGarry & Foster (1981).

CAT I activity was very sensitive to malonyl-CoA inhibition at these low concentrations of palmitoyl-CoA; it is presumed that this was because malonyl-CoA is a competitive inhibitor of CAT I with respect to palmitoyl-CoA (McGarry *et al.*, 1978*a*; Saggerson & Carpenter, 1981*b*). A micromolar concentration of malonyl-CoA was sufficient to produce 60–80% inhibition of CAT I activity in mitochondria from all animals studied except those from starved pregnant rats (see below). However, as shown in Fig. 1, the sensitivity of the enzyme in different mitochondrial preparations varied markedly, depending on the physiological state of the animals from which the mitochondria were isolated. The present data did not enable the calculation of a true K_i value (see, e.g, Dixon & Webb, 1967) for malonyl-CoA. Therefore the concentration of malonyl-CoA required to decrease CAT I activity to 50% of that observed in the absence of the inhibitor ($[\text{malonyl-CoA}]_{0.5}$) was used as an index of the sensitivity of CAT I to malonyl-CoA inhibition. The choice of this parameter enabled an assessment of sensitivity which was independent of any assumptions about the mechanism(s) involved in the observed alterations in sensitivity of CAT I to malonyl-CoA (see below).

The relationship between the sensitivity of CAT I in mitochondria *in vitro* and the malonyl-CoA concentration *in vivo* is shown in Fig. 2. A plot of

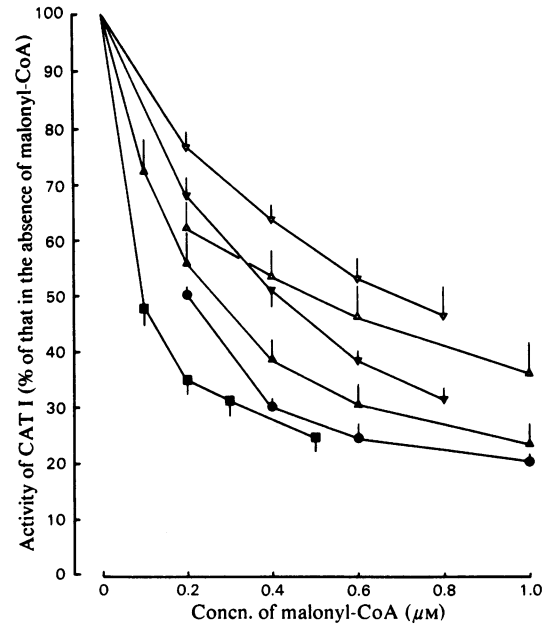

Fig. 1. *Effect of increasing malonyl-CoA concentrations on the activity of CAT I in mitochondria isolated from livers of female rats in various physiological states: virgin* (▽, ▼); *19-day pregnant* (●); *mid-lactating* (△, ▲); *late-lactating* (■)

Filled symbols refer to fed animals, open symbols to 24 h-starved animals. Values are means of three to eight determinations on separate mitochondrial preparations. Vertical bars represent ± S.E.M. The data for mitochondria from starved pregnant rats are not shown in the interests of clarity. The average activity of CAT I in the absence of malonyl-CoA (100%) was 0.30 ± 0.05 nmol/min per mg of mitochondrial protein ($n = 43$) and was not significantly different for mitochondria from animals in different physiological conditions.

values of $[\text{malonyl-CoA}]_{0.5}$ obtained in the present study against malonyl-CoA concentrations in freeze-clamped liver of animals in the same physiological conditions (Zammit, 1981) showed an inverse relationship between the two parameters. Therefore CAT I activity in isolated mitochondria appeared to retain quantitative information about the concentration of malonyl-CoA *in vivo* in the liver from which they were isolated. Interestingly, CAT I was most resistant to malonyl-CoA inhibition in mitochondria from livers of starved pregnant animals (see Fig. 2), such that 50% inhibition could only be obtained at 3 μM-malonyl-CoA. The concentration of malonyl-CoA *in vivo* in livers of starved pregnant animals (0.39 nmol/g wet wt.) is about half that in livers of starved virgin rats (Zammit, 1981). It has

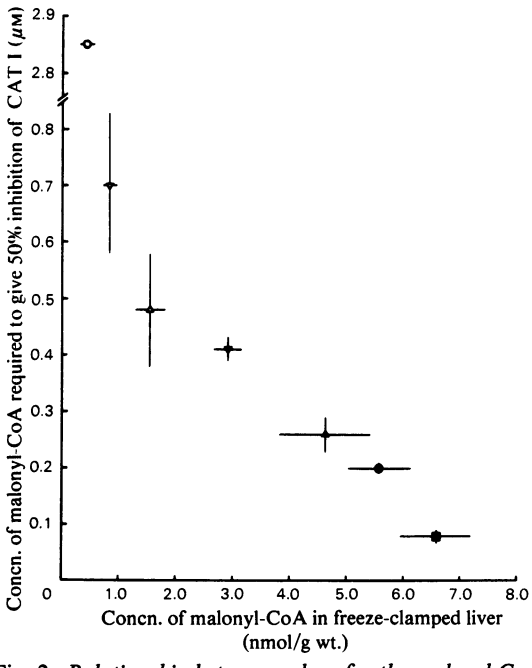

Fig. 2. *Relationship between values for the malonyl-CoA concentration required to produce 50% inhibition of CAT I activity* ($[malonyl\text{-}CoA]_{0.5}$; *see the text*) *in mitochondria from animals in different physiological states (see Fig. 1) and the concentration of malonyl-CoA in freeze-clamped liver (data obtained from Zammit, 1981)*

Values are means ± S.E.M. O, Starved pregnant rats (average of two determinations). Other notations and details are as given in the legend to Fig. 1.

been shown previously that such very low concentrations of malonyl-CoA probably reflect a virtually complete absence of free malonyl-CoA in hepatocytes (McGarry *et al.*, 1978*b*). Therefore the extreme resistance of CAT I to malonyl-CoA inhibition in mitochondria from starved pregnant rats was in consonance with (and an extreme example of) the inverse relationship observed in Fig. 2.

The mechanism(s) through which the quantitative relationship between CAT I sensitivity *in vitro* and the malonyl-CoA concentration *in vivo* is achieved is not known. CAT I could be modified by the same or closely related mechanism(s) which produce the observed changes in malonyl-CoA concentration in the liver, or it could be modified directly (and proportionately to its concentration) by malonyl-CoA itself. Any mechanistic proposal would have to account for the following observations: (i) the CAT I activity in the absence of added malonyl-CoA was virtually identical for all the mitochondrial preparations, and (ii) the 'imprinting' of the enzyme with information about the malonyl-CoA concentration *in vivo*, or a related parameter, was retained throughout the mitochondrial preparation procedure.

The present data constitute strong evidence that the alterations in CAT I sensitivity to malonyl-CoA in mitochondria from starved animals observed previously by other workers (see the introduction) are of physiological importance and represent just a small part of a graded relationship between malonyl-CoA concentrations *in vivo* and sensitivity of CAT I. It is suggested that such a tightly controlled relationship between the two parameters could be a potent amplification mechanism for the reciprocal regulation of the rates of hepatic fatty acid synthesis and oxidation *in vivo* by small changes in cytosolic malonyl-CoA concentration.

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