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Restoration of the properties of carnitine palmitoyltransferase I in liver mitochondria during re-feeding of starved rats

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The recovery of the parameters of the kinetic properties of carnitine palmitoyltransferase (CPT) I in liver mitochondria of starved rats was studied after re-feeding animals for various periods of time. There were no significant changes either in the activity of the enzyme at high palmitoyl-CoA concentrations or in the affinity of the enzyme for palmitoyl-CoA, or in the sensitivity of CPT I to malonyl-CoA inhibition after 3 h or 6 h re-feeding. After 24 h re-feeding, both the affinity of the enzyme for palmitoyl-CoA and the activity of the enzyme were still not significantly different from those for the enzyme in mitochondria from 24 h-starved animals. By contrast, the sensitivity of CPT I to malonyl-CoA inhibition was largely, but not fully, restored to that observed in mitochondria from fed rats.

INTRODUCTION

Three adaptations in the characteristics of hepatic overt carnitine palmitoyltransferase (CPT I) are known to occur after induction of ketogenic states (e.g. diabetes, starvation) in the rat. They are: increased maximal activity (Saggerson & Carpenter, 1981; Zammit *et al.*, 1984), decreased sensitivity to inhibition by malonyl-CoA (Saggerson & Carpenter, 1981; Robinson & Zammit, 1982; Cook, 1984; Brady *et al.*, 1985*a*) and decreased affinity for acyl-CoA substrate (Brady *et al.*, 1985*a*). The first two of these changes would result in increased rates of acylcarnitine synthesis even if there were no changes in the concentrations of substrates or of malonyl-CoA. Therefore, coupled with the increased concentrations of long-chain acyl-CoA (Zammit, 1981) and decreased concentrations of malonyl-CoA (Guynn *et al.*, 1972; Zammit, 1981) that occur in the liver *in vivo* under these conditions, they result in a marked increase in the flux through the reaction catalysed by CPT I. The relative importance of these changes in the induction of the higher intrinsic capacity of the perfused rat liver to oxidize fatty acids, when these are supplied at a fixed concentration in the perfusate (McGarry *et al.*, 1973), is difficult to assess quantitatively. Because they occur concurrently and appear to be inhibited equally by treatment of rats with protein-synthesis inhibitors (e.g. cycloheximide; Saggerson *et al.*, 1984), it has been suggested that the decreased sensitivity of CPT I to malonyl-CoA inhibition may be the result of the synthesis of new enzyme with properties different from those of the enzyme that normally occurs in mitochondria from fed animals (Saggerson *et al.*, 1984; Gamble & Cook, 1985). Similarly, the observations by Brady *et al.* (1985*a*,*b*) that, under conditions that induce increased mitochondrial membrane fluidity, the enzyme is both more active and less sensitive to malonyl-CoA, were interpreted by those authors as indicative of a link between the two parameters.

One method that can be used to assess the relative importance of the different changes in CPT I characteristics and their inter-relationships is to perturb the system acutely in a physiological manner and to study the time courses of the responses of the different parameters in relation to that of the intrinsic change in the ketogenic capacity of the liver (McGarry *et al.*, 1973; Foster, 1984). We have therefore studied the time course of the recovery of the characteristics of CPT I during re-feeding of starved rats. It represents such an acute physiological perturbation. The rapid decline (complete within 2–3 h) in the intrinsic ketogenic capacity of the liver after re-feeding is well documented (McGarry *et al.*, 1973), as is that of the starvation-induced ketonaemia after only 1 h of re-feeding (Holness *et al.*, 1986). On the other hand, the time course of the recovery of the properties of CPT I during the transition from the starved to re-fed state is not known, except for one study in which full recovery of the activity of the enzyme and susceptibility to malonyl-CoA inhibition after 24 h re-feeding were reported (Saggerson *et al.*, 1984).

The results of the present study indicate that there is a minimal acute response of CPT I to re-feeding of starved rats, represented by a further decline in the affinity of the enzyme for acyl-CoA substrate. Changes in the susceptibility of the enzyme to malonyl-CoA inhibition occur remarkably slowly and take place in the absence of any significant decrease in enzyme activity during the first 24 h of re-feeding.

MATERIALS AND METHODS

Animals

Female Wistar rats (A. Tuck and Sons, Basingstoke, Hants., U.K.) were maintained for at least 2 weeks on a 12 h-dark–12 h-light cycle; lights were switched on at 09:00 h. Fed rats received food *ad libitum*. Starved rats had food removed at 09:00 h and were used 48 h later; re-fed rats had food re-introduced at this time and were allowed to feed *ad libitum*, for the number of hours indicated, before being used. The average weight of food consumed by the rats was 5.0 g after 1 h and 6.3 g after 3 h re-feeding. Total blood ketone-body concentrations were decreased from 1.41 ± 0.20 μmol/ml in starved rats to 0.32 ± 0.09 and 0.16 ± 0.04 μmol/ml in 1 h- and 3 h-re-fed rats respectively.

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

Preparation of mitochondria and assay of CPT I activity

Liver mitochondria were prepared as described previously (Zammit & Corstorphine, 1985; Grantham & Zammit, 1986), except that livers were excised from rats that had been anaesthetized 20 min previously with pentobarbital (60 mg/kg). Mitochondria were finally suspended in a medium containing 150 mM-KCl, 5 mM-Tris and 1 mM-EGTA (pH 7.4 at 0 °C), and kept at 0 °C until used (within 30 min). Assays were performed in a medium containing 150 mM-KCl, 5 mM-Tris, 1 mM-EGTA, 1 mM-dithiothreitol, 3 mM-L-[*methyl*-^{3}H]carnitine (0.03 Ci/mol), 10 mg of albumin/ml, 1 μg of rotenone/ml and 1 μg of antimycin A/ml. Additions of palmitoyl-CoA and malonyl-CoA were made as indicated in the legends to the Figures. The final volume was 2.0 ml and the pH was 7.4 at 37 °C. Samples (50 μl) of mitochondrial suspension were added to the reaction medium, from which L-carnitine was omitted, and incubated at 37 °C for 2 min. The reactions were started by addition of 50 μl of a prewarmed carnitine solution and stopped after 2 min by addition of 0.3 ml of 6 M-HCl. Incorporation of [^{3}H]carnitine into butanol-soluble radioactivity was quantified as described previously (Robinson & Zammit, 1982). The protein content of mitochondrial preparations was measured by the method of Lowry *et al.* (1951), with bovine albumin as standard.

Materials

The sources of these were as described previously (Robinson & Zammit, 1982), except that [^{3}H]carnitine was synthesized as described by Stokke & Bremer (1969).

Expression of results

Because of the complexity of the interactions between the monomolecular, micellar and protein-bound forms of palmitoyl-CoA in solution (Bartlett *et al.*, 1985), the data obtained in the presence of different amounts of palmitoyl-CoA are plotted against total palmitoyl-CoA concentration. At concentrations higher than 540 nmol/ml, the CPT activity increased sharply. This was presumed to be due to the detergent effects of palmitoyl-CoA in exposing latent CPT (CPT II) activity. Plots of CPT I velocity versus total palmitoyl-CoA concentration could not be linearized by either double-reciprocal or Hill plots of the data. Consequently, plots in which the affinity of CPT I for palmitoyl-CoA under different conditions is compared are normalized by expressing them as a percentage of the activity obtained at the highest concentration of palmitoyl-CoA used (540 nmol/ml).

RESULTS AND DISCUSSION

Starvation of the rats resulted in the changes in the CPT I kinetic characteristics that have been reported previously, namely an increased activity in the absence of malonyl-CoA (Saggerson & Carpenter, 1981; Zammit *et al.*, 1984), increased $K_{0.5}$ for palmitoyl-CoA (Brady *et al.*, 1985*a*) and increased $I_{0.5}$ for malonyl-CoA at a fixed (130 μM) palmitoyl-CoA concentration (Saggerson & Carpenter, 1981; Robinson & Zammit, 1982). It is noteworthy that the use of saturating carnitine concentrations (K_m for carnitine is 230 μM; see also Brady *et al.*, 1985*a*) allowed us to eliminate the possible interference

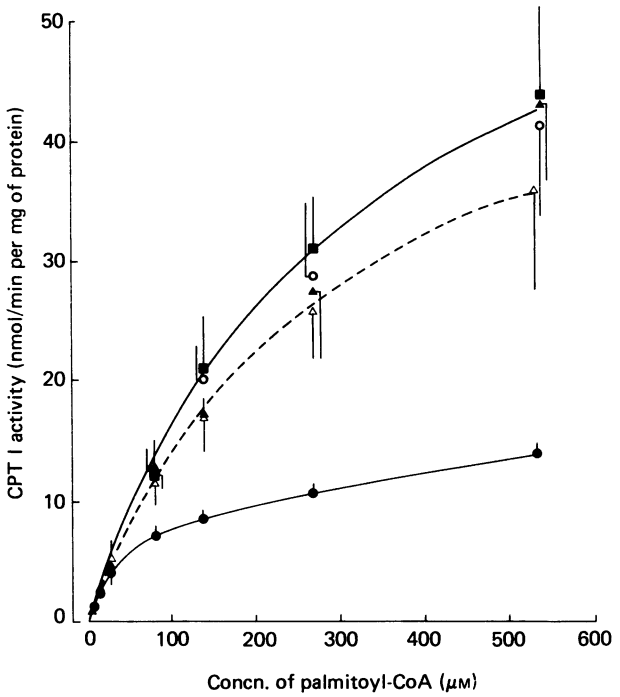

Fig. 1. Effect of starvation and re-feeding of rats on the activity of CPT I at different concentrations of palmitoyl-CoA in liver mitochondria

Rats were either fed *ad libitum* (●) or starved for 48 h (○) and then re-fed for 3 h (▲), 6 h (■) or 24 h (△). Values are means ± S.E.M. for four to six determinations on separate mitochondrial preparations.

of changes in the K_m for carnitine in the presence of malonyl-CoA (Bird & Saggerson, 1985). The K_m for L-carnitine found in the present study was higher than that reported by Mills *et al.* (1984), but similar to that reported by Brady *et al.* (1985*a*). The reasons for this difference in apparent affinities for carnitine observed in different laboratories are not known, but it is possible that the disparate conditions used for assay of CPT I activity may be a contributory factor.

Re-feeding starved rats for 3 h resulted in no change in either the activity of CPT I at high palmitoyl-CoA concentration or the $I_{0.5}$ value for malonyl-CoA (Figs. 1 and 3). These observations were unexpected, on two counts. Firstly, this period of re-feeding completely reverses the increased intrinsic ketogenic capacity of perfused rat liver observed in starvation, to values measured for perfused livers obtained from fed animals (McGarry *et al.*, 1973); it is also sufficient to produce a marked decrease in blood ketone-body concentration (see the Materials and methods section). Secondly, it has been reported (Gamble & Cook, 1985) that acute treatment of diabetic rats with insulin resulted in a decrease in apparent K_i for malonyl-CoA within 1 h and complete reversal of the effects of diabetes on this parameter within 4 h. The present data suggest that acute changes do not occur either in the amount of CPT I (assuming constant specific activity) or in the susceptibility of the enzyme to malonyl-CoA inhibition, when insulin is increased by re-feeding. Even after 6 h re-feeding, the malonyl-CoA inhibition curve was not significantly different from that observed for the enzyme in mitochondria obtained from starved rats.

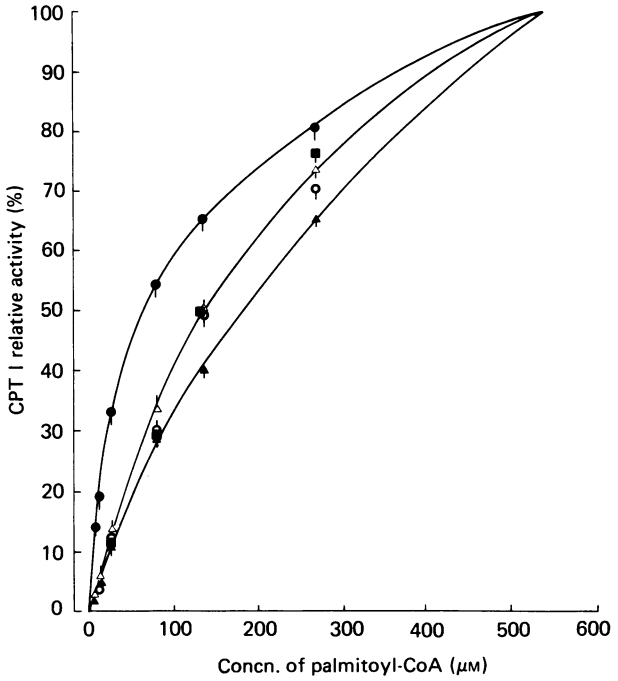

Fig. 2. Effect of starvation and re-feeding of rats on the affinity of CPT I for palmitoyl-CoA

See the legend to Fig. 1 for details. Values are means ± S.E.M. of the activities of CPT I expressed as a percentage of that obtained at 540 μM-palmitoyl-CoA for the same preparation. The number of determinations on separate preparations was four to six in each instance.

After 24 h re-feeding, there was only a marginal decrease in the activity of CPT I. Moreover, the sensitivity of the enzyme to malonyl-CoA inhibition was still not totally restored to the level seen in mitochondria from fed animals (Fig. 3).

The only acute change observed in the properties of CPT I during the first 3 h of re-feeding was a further small decrease (in addition to that induced by starvation) of the affinity of the enzyme for palmitoyl-CoA (Fig. 2). Although this further decrease in affinity was small, it was reproducibly observed.

The decreased affinity of CPT I for acyl-CoA substrate in mitochondria from starved rats (in which the effects of glucagon are likely to predominate) agrees with the observation made by Brady *et al.* (1985*a*). It raises the question as to the physiological significance of the reported effects of glucagon in increasing the affinity of CPT for acyl-CoA through increased phosphorylation in hepatocytes incubated with the hormone *in vitro* (Harano *et al.*, 1985). In the latter study, no distinction was made between CPT I and CPT II and the enzyme(s) was (were) solubilized from the mitochondrial inner membrane. The present results suggest that the conclusions drawn from that study may not be applicable to the liver *in vivo*, although it should be stressed that no attempt was made in the present study to preserve the putative phosphorylation state of CPT I during mitochondrial preparation.

The physiological significance of the decreased affinity for acyl-CoA after starvation may reside in the facilitation of the rapid decline in intrinsic ketogenic capacity of the liver after re-feeding. Thus, although it is unlikely to affect the ability of the liver to increase the flux through CPT I under conditions of increased delivery of fatty acids to the liver (starvation), it could represent a mechanism whereby, on re-feeding, the effects of decreased acyl-CoA concentration would be amplified. This would contribute towards the rapid decline in the ketogenic capacity of the liver during the first 3 h of re-feeding. No reversal of the effect was observed during 24 h of subsequent re-feeding. Since both the absolute malonyl-CoA concentrations (Foerster *et al.*, 1979; Lynen, 1979) and the sensitivity of CPT I to malonyl-CoA inhibition (the present paper) recover very slowly after re-feeding, the malonyl-CoA effect on CPT I may not be sufficiently great, during the initial stages of re-feeding, to play a prominent role in the acute decline in ketogenic capacity. Under these conditions the retention of low affinity by CPT I for its substrate can be rationalized in the light of the observation that decreased flux through CPT I has to be achieved in the presence of a highly elevated activity of the enzyme, which did not decrease significantly even after a 24 h re-feeding period (Fig. 1).

The slowness of the recovery of the properties of CPT I, especially of its sensitivity to malonyl-CoA inhibition, is not unique among the responses of liver metabolism to re-feeding of starved rats. Thus it is well established that, on re-feeding, high rates of gluconeogenesis persist for a considerable time (Sugden *et al.*, 1983; Newgard *et al.*, 1984). In particular, the recovery of fructose 2,6-bisphosphate concentrations only starts after 5 h of

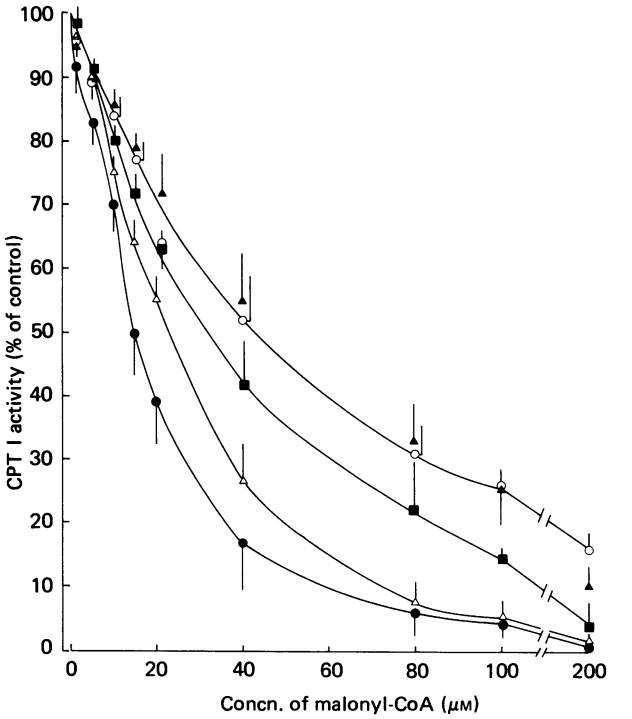

Fig. 3. Effect of starvation and re-feeding of rats on the sensitivity of CPT I to malonyl-CoA inhibition in liver mitochondria

See the legend to Fig. 1 for details. Values are means ± S.E.M. of activities expressed as a percentage of that obtained at 130 μM-palmitoyl-CoA in the absence of malonyl-CoA. The number of determinations on separate mitochondrial preparations was four to six in each instance.

re-feeding (Kuwajima *et al.*, 1984) and is not complete until much later. It is noteworthy that the concentration of malonyl-CoA (and by inference the rate of lipogenesis) is very sluggish in recovering to the values observed in the livers of fed animals (Lynen, 1979; Foerster *et al.*, 1979). This agrees with the observations that re-feeding of starved rats does not result in increased hepatic lipogenesis during the first 2 h, although it increases lipogenesis in other tissues (Agius & Williamson, 1981; Sugden *et al.*, 1981). More recently, this phenomenon has been shown to coincide with a prolonged continued inactivation of hepatic pyruvate dehydrogenase by phosphorylation during the initial stages of re-feeding (Holness *et al.*, 1986). In this respect it would appear that the time course of the re-establishment of the sensitivity of CPT I to malonyl-CoA inhibition is more similar to that of the recovery of hepatic concentration of malonyl-CoA and of lipogenesis than to that of the decrease in the intrinsic ketogenic capacity of the liver.

The present data also have important implications for the more mechanistic aspects of the regulation of CPT I. Evidently, the various properties of CPT I are capable of independent modulation. Significant restoration of the sensitivity of the enzyme to malonyl-CoA can occur in the absence of a significant decrease in the activity of CPT I at high palmitoyl-CoA concentrations after 24 h re-feeding of starved rats (Figs. 1 and 2). This observation is difficult to reconcile with the suggestions that the decreased sensitivity of CPT I to malonyl-CoA inhibition that occurs on starvation (Saggerson *et al.*, 1984) or induction of diabetes (Gamble & Cook, 1985) is obligatorily related to the synthesis of new enzyme. It is possible that changes in the properties of CPT I under these conditions are due to an alteration in the association of the enzyme with the mitochondrial inner membrane (Zammit & Corstorphine, 1985) and, as suggested previously (Zammit, 1986), may be related to changes in the composition of the latter and to the fluidity differences observed between membranes of mitochondria from fed and starved rats (Brady *et al.*, 1985*a*).

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