EFFECTS OF FASTING AND MALONYL CoA ON THE KINETICS OF CARNITINE PALMITOYLTRANSFERASE AND CARNITINE OCTANOYLTRANSFERASE IN INTACT RAT LIVER MITOCHONDRIA

E. David SAGGERSON and Carol A. CARPENTER
Department of Biochemistry, University College London, Gower Street, London WC1E 6BT, England

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1. Introduction

There has been considerable interest in the observation that the overt form of carnitine palmitoyltransferase (CPT_1) in liver mitochondria is potently inhibited by malonyl CoA [1,2]. It has been suggested [2] that this is a competitive type of inhibition against long chain acyl CoA substrates. However, this is based upon measurements of ketogenesis [3–5] or indirect calculations of CPT_1 activity [1]. No studies have been performed in which CPT_1 activity has been measured directly in intact mitochondria at various concentrations of the carnitine and acyl CoA substrates. Accordingly this is undertaken here, using liver mitochondria from both fed and fasted rats since the sensitivity of CPT_1 to malonyl CoA is decreased in the fasted state [6]. The use of intact mitochondria is important both to avoid measurement of CPT_2 which is insensitive to malonyl CoA [1] and to avoid disruptive procedures which may remove CPT_1 from mitochondrial membranes and render the enzyme malonyl CoA-insensitive [1].

Rat liver mitochondria also appear to contain an overt carnitine octanoyltransferase (COT) [7,8] of unknown physiological function which is also inhibited by malonyl CoA [6]. The effects of this inhibitor upon COT and CPT_1 differ in that the former is more sensitive to malonyl CoA and this sensitivity is not diminished by fasting [6]. It was therefore also of interest to investigate the effects of malonyl CoA upon the kinetics of COT.

2. Materials and methods

Sources of chemicals are described in [6]. Male Sprague-Dawley rats of 165–175 g were selected 1 day prior to sacrifice and then either fed as usual or fasted for 24 h starting at ~10:00 h. Livers were homogenised and centrifuged to obtain mitochondria as in [6]. CPT_1 and overt COT activities were measured in fresh whole mitochondria by following the incorporation of [*methyl*-^{3}H]carnitine into butanol-soluble products [6]. Rates were linear with time and proportional to amount of mitochondrial protein which was normally present at ~200 mg/ml assay mixture.

3. Results and discussion

Fig.1(a) shows that there is a sigmoidal relationship between CPT_1 activity and palmitoyl CoA concentration giving a $K_{0.5}$ for this substrate of ~30 μM (the assay contained 1.3 mg defatted albumin/ml). Malonyl CoA increased this sigmoidicity, but had no appreciable effect upon V_{max}. In the presence of 50 μM malonyl CoA the $K_{0.5}$ for palmitoyl CoA was approximately doubled. The sensitivity of CPT_1 to the regulatory effects of malonyl CoA is therefore clearly dependent upon the concentration of the acyl CoA substrate. After a fasting period of 24 h there was little change in the $K_{0.5}$ for palmitoyl CoA in the absence of malonyl CoA (fig.1b) but, as shown in [6], fasting caused a diminution in the inhibitory effect of malonyl CoA.

As shown by Lineweaver-Burke analysis (fig.2) the relationship between CPT_1 activity and carnitine concentration was hyperbolic over the tested range (20–400 μM L-carnitine) and the K_m for carnitine (91 μM) was unaltered by malonyl CoA or by fasting.

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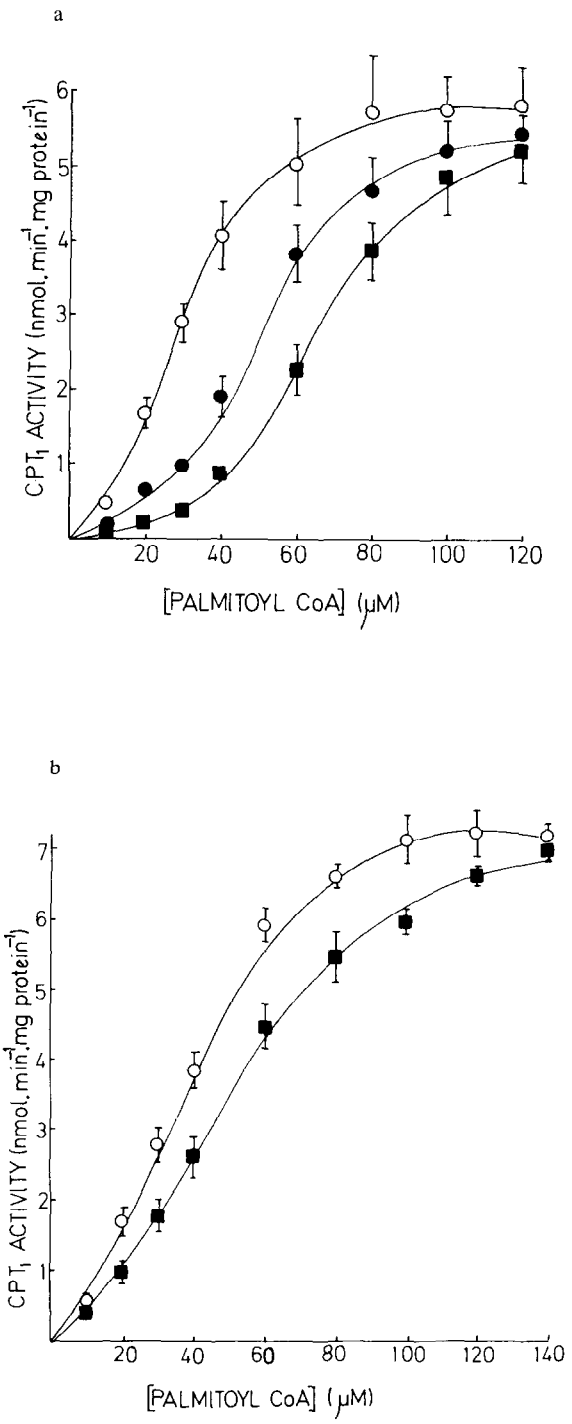

Fig.1. Effects of palmitoyl CoA and malonyl CoA on CPT_1 activity. L-Carnitine was present throughout at 400 μM: (○) [malonyl CoA] = 0; (●) [malonyl CoA] = 10 μM; (■) [malonyl CoA] = 50 μM; (a) livers from fed rats; (b) livers from fasted (24 h) rats. The values are means ± SEM of 4 expt.

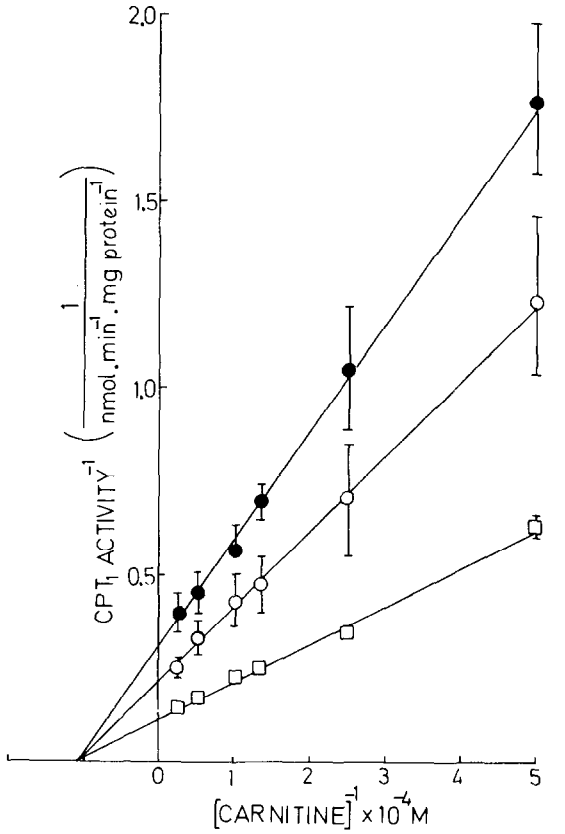

Fig.2. Lineweaver-Burke plot showing dependence of CPT_1 activity on L-carnitine concentration. Palmitoyl CoA was present throughout at 40 μM: (○) fed state, [malonyl CoA] = 0; (●) fed state, [malonyl CoA] = 10 μM; (□) fasted state, [malonyl CoA] = 0. The values are means ± SEM of 4 expt.

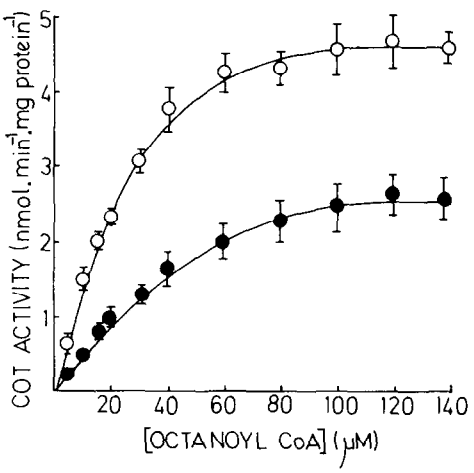

Fig.3. Effects of octanoyl CoA and malonyl CoA on COT activity. Mitochondria were obtained from fed rats. L-Carnitine was present throughout at 400 μM: (○) [malonyl CoA] = 0; (●) [malonyl CoA] = 4 μM. The values are means ± SEM of 5 expt.

Fig.3 shows that, unlike CPT_1 there was no appreciable sigmoidicity in the relationship between COT activity and the concentration of acyl CoA substrate. Also, the inhibitory effect of malonyl CoA upon this activity could not be overcome by increasing the concentration of the acyl CoA substrate.

These findings are noteworthy in that firstly they demonstrate sigmoidal kinetics for CPT_1 in intact mitochondria which might be suggestive of allosteric properties and secondly they show further differences between the properties of CPT_1 and overt COT.

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