Malonyl CoA inhibition of carnitine palmityltransferase in rat heart mitochondria

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The effects of malonyl CoA on carnitine palmityltransferase I (CPT-I), fatty acid-supported state 3 respiration, and carnitine reversal of palmityl CoA inhibition of state 3 respiration and of the adenine nucleotide translocator, were studied in isolated rat heart mitochondria. Malonyl CoA was a potent competitive inhibitor of CPT-I with an I_{50} of 0.8 μM. Fasting did not affect CPT-I activity or the I_{50} value of malonyl CoA. Malonyl CoA inhibited fatty acid-supported respiration and prevented carnitine from reversing the inhibition of the adenine nucleotide translocator by palmityl CoA. These findings suggest that malonyl CoA may affect fatty acid oxidation in the heart.

Malonyl CoA *Carnitine* *Palmityltransferase* *Mitochondria* *Fasting heart*

1. INTRODUCTION

Malonyl CoA is a potent inhibitor of carnitine palmityltransferase-I (CPT-I), which catalyzes the reaction:

carnitine + palmityl CoA ⟶

palmitylcarnitine + CoA

In the liver, it has been suggested that this reaction may regulate hepatic fatty acid oxidation and ketogenesis [1–3]. In fasted animals, hepatic malonyl CoA levels decrease, CPT-I activity is increased [4], and the inhibitory effect of malonyl CoA on fatty acid oxidation is decreased [5]. Malonyl CoA will also inhibit CPT-I in a number of extrahepatic tissues, including the heart [6]. Malonyl CoA is found in measurable amounts in heart and kidney; the levels decrease during fasting [3,7]. However, the role of malonyl CoA–CPT-I interaction in cardiac tissue is unclear. It is not known if CPT-I sensitivity to malonyl CoA changes in fasting or whether malonyl CoA will actually affect mitochondrial oxidation of fatty acids by heart mitochondria.

Here, we determined the effects of malonyl CoA on (i) CPT-I activity of heart mitochondria isolated from fed and fasted rats, (ii) fatty acid-supported state 3 respiration, and (iii) carnitine reversal of palmityl CoA inhibition of state 3 respiration and the adenine nucleotide translocator.

2. MATERIALS AND METHODS

Rat heart mitochondria were isolated in medium containing 250 mM sucrose, 4 mM Tris (pH 7.4), 1 mM EGTA, and 0.2% fatty acid-free bovine serum albumin [8]. Mitochondrial protein was measured by the biuret method [9], and respiratory activity determined on a Gilson 5/6 oxygraph [10]. Adenine nucleotide translocator activity was quantitatively determined by measuring the slower forward uptake of [^{14}C]ATP at 2°C, using the carboxyatractyloside inhibitor stop technique [8]. CPT-I activity was assayed using an isotope for-

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ward reaction similar to that described in [11]. Approx. 30 mg of muscle was homogenized in 1 ml of isolation medium (300 mM sucrose, 5 mM Mops (pH 7.2) and 2 mM EDTA (pH 7.4)). A 0.05-ml aliquot was removed and analyzed for non-collagenous protein. Another aliquot was freeze–thawed 3 times to disrupt mitochondrial membranes. 50- and 100-μl aliquots were added to an incubation mixture containing 80 mM KCl, 50 mM Mops (pH 7.2), 4 mM dithiothreitol, 1 mg/ml albumin, 0.2 mM L-carnitine, 50 μM palmityl CoA, and 0.78 μCi DL-[^{14}C]carnitine. The assay was run at 30°C for 7 min and terminated by adding 0.1 ml concentrated HCl. Palmitylcarnitine was extracted with 0.9 ml of butanol-saturated water and 1 ml butanol. The solution was vortex mixed and centrifuged. A 0.75-ml portion was removed from the upper phase and extracted again. The radioactivity in a 0.5-ml aliquot from this upper phase was counted using a Packard Tri-Carb liquid scintillation spectrometer.

3. RESULTS AND DISCUSSION

The effect of palmityl CoA and carnitine on rat heart mitochondrial CPT-I activity is shown in fig.1. In the absence of malonyl CoA, the estimated K_m values for palmityl CoA and carnitine were 18 μM and 0.6 mM, respectively. In the presence of 5 μM malonyl CoA, CPT-I activity was severely inhibited at the lower palmityl CoA concentrations and the substrate saturation curve for palmityl CoA became sigmoid. At higher concentrations of palmityl CoA (60 μM), malonyl CoA (5 μM) was not inhibitory, suggesting that the inhibition was competitive. The sigmoid curve probably resulted from the interaction of varying amounts of palmityl CoA with the constant amount of bovine serum albumin in the reaction mixture. Malonyl CoA inhibited the carnitine substrate curve at all concentrations, suggesting that malonyl CoA does not competitively interact with carnitine.

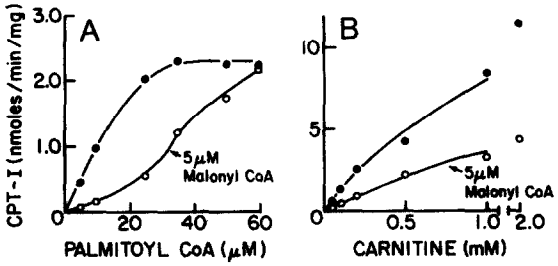

Fig.1. Effect of palmityl CoA and carnitine concentration on carnitine palmityltransferase I (CPT-I) with and without 5 μM malonyl CoA in isolated rat heart mitochondria. (A) Palmityl CoA concentration curve, with carnitine at 0.2 mM. (B) Carnitine concentration curve, with palmityl CoA at 50 μM.

Fig.2 shows the effect of varying malonyl CoA concentrations on the activity of CPT-I in heart mitochondria isolated from fed and 48-h-fasted rats. In contrast to results for liver [4,5], fasting did not increase CPT-I activity nor did it affect malonyl CoA inhibition of CPT-I. The I_{50} value for heart mitochondria isolated from fed and fasted rats was 0.8 μM. This value is slightly higher than the I_{50} for rat heart mitochondria found in [7], but it is still within the concentration range for malonyl CoA levels found in the heart.

The effect of malonyl CoA on rat heart mitochondrial respiration is shown in table 1.

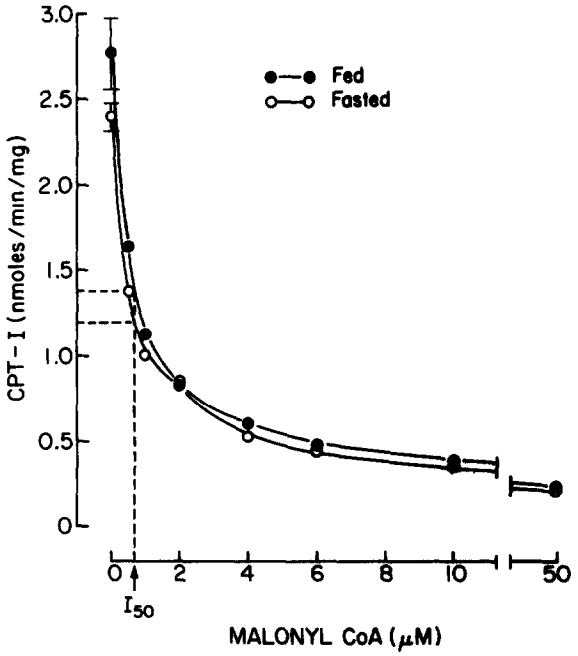

Fig.2. Effects of fasting on carnitine palmityltransferase-I (CPT-I) sensitivity to inhibition by malonyl CoA in isolated rat heart mitochondria. Rats were fasted for 48 h. Palmityl CoA and carnitine concentrations were 50 μM and 0.2 mM.

Table 1

Effects of malonyl CoA on rat heart mitochondria state 3 respiration

Substrate	Malonyl CoA concentration (μM)					
	0	0.5	1.0	5.0	10.0	50.0
Pyruvate + malate	314	–	–	–	–	313
Pyruvate + malate + 5 μM palmityl CoA	90	80	80	100	80	79
Pyruvate + malate + 5 μM palmityl CoA + 1 mM L-carnitine	274	210	202	176	192	180
5 μM palmityl CoA + malate + 2 mM L-carnitine	144	–	94	58	72	36

Results are expressed as natom oxygen/mg per min

Malonyl CoA had no effect on pyruvate- and malate-supported state 3 respiration or on palmityl CoA (5 μM) inhibition of this respiration. It did, however, prevent carnitine (1 mM) from reversing palmityl CoA inhibition. The effect of malonyl CoA was seen at a concentration as low as 0.5 μM, but the most effective concentration was 5 μM. Presumably, malonyl CoA inhibited CPT-I, which would prevent carnitine from reacting with palmityl CoA to produce palmitylcarnitine plus free CoA. Palmityl CoA has been shown to inhibit the mitochondrial adenine nucleotide translocator [12,13], the transport protein that facilitates the exchange transport of cytosolic ADP for intramitochondrially produced ATP [14]. It has been suggested to be one of the key steps and possibly the rate-limiting step in oxidative phosphorylation. Palmitylcarnitine has no effect on this transport protein [12,13]. Table 1 also shows the effects of malonyl CoA on palmityl-CoA malate carnitine-supported state 3 respiration. The rate of respiration was less with these substrates than with pyruvate plus malate. Increasing concentrations of malonyl CoA produced a progressive decrease in oxygen uptake.

We also determined the effect of palmityl CoA, carnitine, and malonyl CoA on a specific assay for the adenine nucleotide translocator (table 2). Addition of 10 μM palmityl CoA inhibited this reaction by 32%. Carnitine (2 mM) partially reversed the inhibition, but 50 μM malonyl CoA completely prevented this reversal.

In summary, these results show that malonyl CoA does not affect adenine nucleotide translocator activity, but it is a potent and relatively specific inhibitor of CPT-I and fatty acid-supported respiration of rat heart mitochondria. The malonyl CoA–CPT-I interaction may also be important in controlling mitochondrial respiration because of its effect on carnitine reversal of palmityl CoA inhibition of the adenine nucleotide translocator. In contrast to liver CPT-I, heart CPT-I shows a sensitivity to malonyl CoA that is

Table 2

Effects of malonyl CoA on carnitine reversal of palmityl CoA inhibition of the mitochondrial adenine nucleotide translocator

Palmityl CoA (μM)	Carnitine (mM)	Malonyl CoA (μM)	Adenine nucleotide translocator (nmol/mg per min)[a]
0	0	0	3.00
5	0	0	2.78
5	2	0	2.90
5	2	50	2.76
10	0	0	2.04
10	2	0	2.25
10	2	50	2.08

[a] Values are the average of 2 experiments performed in triplicate

not affected by fasting. The levels of malonyl CoA that produce these effects are within the in vivo concentrations [7]. Although the precise role of malonyl CoA in the heart is still unclear, these results provide further evidence that malonyl CoA may exert a regulatory effect on fatty acid oxidation in the heart. Since fatty acids are the preferred substrate for the heart [15], this reaction may be of particular importance.

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