ACETYLCARNITINE METABOLISM IN BLOWFLY FLIGHT MUSCLE MITOCHONDRIA

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1. Introduction

Carnitine is involved in the movement of acetyl-CoA and fatty acyl-CoA into the matrices of mammalian mitochondria, where they are oxidized [1]. In some insects, carnitine may have a different role.

Blowfly flight muscle mitochondria will not oxidize palmitoylcarnitine as they do not contain the enzymes for fatty acid oxidation [2,3], and they cannot oxidize acetyl-CoA in the presence of carnitine as there is no carnitine acetyltransferase activity between the membranes. However, there is transferase activity in the matrix and acetylcarnitine is oxidized rapidly [3]. Carnitine acetyltransferase is not membrane-bound in flight muscle mitochondria, therefore carnitine and acetyl carnitine have to cross the membrane to react with the enzyme. It was postulated that this was unnecessary in rat liver mitochondria as the transferase is probably attached to the inner mitochondrial membrane [4].

This paper reports some features of acetylcarnitine metabolism by blowfly flight muscle mitochondria. Evidence is presented for the existence of a carrier for acetylcarnitine. Recently [5,6] it has been shown that ox heart mitochondria contain L-carnitine which can be exchanged 1 for 1 with external carnitine. The carrier for acetylcarnitine in flight muscle mitochondria does not involve such an exchange.

2. Method and materials

Flight muscle mitochondria were prepared from *Calliphora erythrocephela* blowflies by the method of Chappell and Hansford [7] using the proteinase Nagarse. However, the isolation medium used was 320 mM sucrose, 1 mM EGTA and 5 mM Tris–HCl, pH 7.4 at 20°C. The blowfly pupae were generously supplied by the A.R.C. Unit of Invertebrate Chemistry and Physiology at the University of Sussex.

Oxygen consumption was measured using a Clark-type oxygen electrode as previously described [8]. DL-acetylcarnitine was obtained from Sigma Chemical Co., St. Louis, Mo., USA. (−)-Carnitine was obtained from Koch-Light Labs., Colnbrook, Bucks, England.

3. Results

Blowfly flight muscle mitochondria prepared using Nagarse are unable to oxidize pyruvate well as they lack endogenous substrates [9]. Proline is usually used to 'prime' pyruvate or acetylcarnitine oxidation since alone it accounts for little or no oxygen consumption, but in the presence of pyruvate or acetylcarnitine it can provide glutamate and hence oxaloacetate to react with acetyl CoA [10]. For maximum uncoupled oxidation it is necessary to add ADP as well as uncoupler to flight muscle mitochondria to activate the NAD^+-linked isocitrate dehydrogenase [11]

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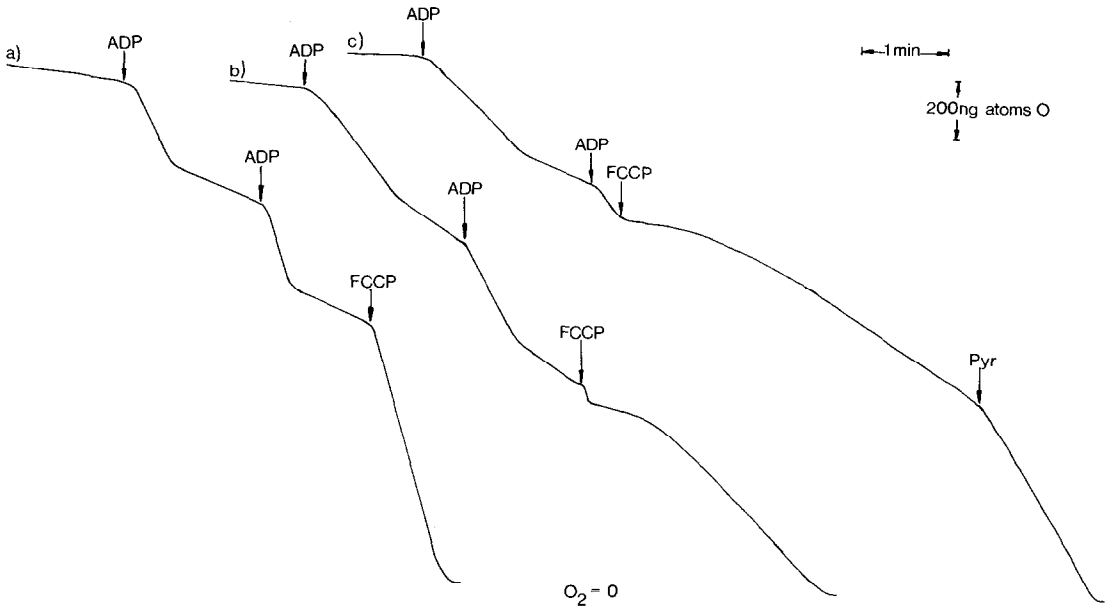

Fig.1. The effect of uncoupler on pyruvate and acetylcarnitine oxidation. Flight muscle mitochondria (3 mg) were added to a medium containing 150 mM KCl, 10 mM Tris–HCl, 30 mM potassium phosphate, pH 7.2 and 10 μg/ml BSA. The following substrates were then added (a) pyruvate (3.7 mM) and proline (3.7 mM), and (b) and (c) L-acetyl carnitine (2.5 mM) and proline (3.7 mM). The total vol was 4 ml and the temperature was 25°C. Where indicated ADP was added to give 0.25 mM, FCCP to give 10 μM and pyruvate to give 3.7 mM.

Fig.1a shows that when *p*-trifluoromethoxy-carbonylcyanidephenylhydrazone (FCCP) was added to flight muscle mitochondria oxidizing pyruvate in state 4 a rapid rate of oxidation was initiated which only ceased when the system became anaerobic. This is the usual effect of adding uncoupler to mitochondria oxidizing a substrate. Fig.1b shows the effect of FCCP on mitochondria oxidizing acetylcarnitine in State 4. Initially there was a rapid rate of oxidation, but then oxidation ceased and then gradually increased again without further additions. The addition of FCCP to mitochondria oxidizing acetylcarnitine in State 3 caused an immediate inhibition in the rate of oxidation followed by a gradual increase. This effect is seen at all acetylcarnitine concentrations used (up to 10 mM DL acetylcarnitine) and at various FCCP concentrations (2–10 μM).

It was found that 10 μM FCCP in the presence of 10 μg BSA/ml had no effect on carnitine acetyltransferase activity in Triton-treated mitochondria. Therefore the FCCP-induced changes in acetylcarnitine oxidation are probably an effect on the membrane permeability of acetylcarnitine.

When the experiments in fig.1 were repeated so that the change in optical density at 610 nm could be observed, it was found that the mitochondria were swelling gradually during the time when the rate of oxidation gradually increased after the inhibition. It is possible that this increase in oxidation is due to acetylcarnitine leaking into the matrix, as the mitochondria swell.

Fig.2 shows the effect of carnitine on mitochondria oxidizing acetylcarnitine in State 3. It has been found that aspartate, like proline, will 'prime' pyruvate or acetylcarnitine oxidation in these mitochondria (S. M. Danks and J. D. McGivan, unpublished observations) by providing oxoaloacetate in the matrix. Carnitine inhibits acetylcarnitine oxidation by isolated mitochondria. A plot of the carnitine concentration against the reciprocal of the rate of oxidation was curved indicating that the inhibition was partially competitive [12].

It was found that in Triton-treated mitochondria the rate of metabolism of acetylcarnitine by carnitine

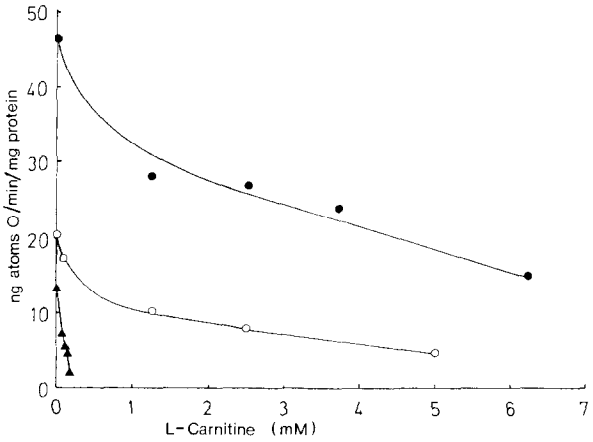

Fig.2. The effect of carnitine on acetylcarnitine oxidation. Flight muscle mitochondria (3 mg) were added to a medium containing 150 mM KCl, 10 mM Tris–HCl, 30 mM potassium phosphate, pH 7.2 and 10 μg/ml BSA. The rate of oxygen consumption was measured after the addition of DL-acetylcarnitine, 10 mM potassium aspartate, 0.25 mM ADP and varying concentrations of carnitine. The total vol. was 3 ml and the temperature was 25°C. (•) 1 mM DL-acetylcarnitine; (○) 0.5 mM DL-acetylcarnitine; (▲) 0.33 mM DL-acetylcarnitine.

acetyltransferase was not inhibited by carnitine. Therefore the carnitine is probably competing with the acetylcarnitine for a particular site on the mitochondrial membrane. Under the conditions described in fig.2 the movement of acetylcarnitine across the membrane is limiting its rate of oxidation.

Although flight muscle contains a large amount of carnitine there is no carnitine in the mitochondrial matrix [3]. It was found from enzymatic assay that flight muscle mitochondria as prepared contain 3–4 nmol acetylcarnitine/mg mitochondrial protein. This is similar to the amount of carnitine found in ox heart mitochondria [5].

4. Discussion

Uncoupler does not usually inhibit oxidation under the conditions described in fig.1, however this has been shown before by Levitsky and Skulachev [13]. They found that palmitoylcarnitine oxidation by rat liver mitochondria was inhibited by uncoupler. Palmitoylcarnitine alters the electric conductance of an artificial phospholipid membrane and the change was due to the transmembrane diffusion of cationic palmitoylcarnitine [13]. Neither acetylcarnitine nor carnitine affected the electric conductance of an artificial phospholipid membrane, therefore their transfer across the flight muscle mitochondrial membrane is more likely to be carrier mediated. This is supported for acetylcarnitine by the evidence that carnitine inhibits acetylcarnitine oxidation, but does not affect the rate of metabolism through carnitine acetyltransferase.

The inhibition of flight muscle mitochondrial acetylcarnitine oxidation by uncoupler could be explained by involking an energy-linked transfer of acetylcarnitine into the matrix driven by an electrochemical gradient of H^+ [14]. The movement of positively charged acetylcarnitine [15] into the matrix would be favoured by the membrane potential (negative on the matrix side of the membrane). Then the addition of uncoupler to mitochondria oxidizing acetylcarnitine caused a rapid oxidation of any acetylcarnitine in the matrix (fig.1b) but then transport and therefore oxidation ceased (fig.1b and 1c).

Initially an antiporter was postulated [16], exchanging acetylcarnitine for carnitine, similar to that for glutamine/glutamate [17]. However, the endogenous acetylcarnitine cannot be exchanged for externally-added, radiolabelled carnitine or acetylcarnitine under the conditions described for the exchange in ox heart mitochondria [6].

The mechanism by which carnitine enters the matrix is unclear, it may diffuse across the membrane as a zwitterion. This has been postulated to occur for several neutral amino acids in rat heart and rat brain mitochondria [18]. The physiological significance of the energy-driven transport of acetylcarnitine into the matrix is not known. It has been postulated [3] that in flight muscle carnitine may act as a store for active acetyl groups under oxaloacetate-limiting conditions. The carrier would probably transfer active acetyl groups into the matrix when oxaloacetate is freely available. This is being investigated further.

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