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Palmitoyl-CoA inhibits the mitochondrial inner membrane anion-conducting channel

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Palmitoyl-CoA is shown here to inhibit the pH-dependent anion-conducting channel (IMAC) in the inner membrane of rat liver mitochondria, with half-maximal inhibition at 2.4 μM. It has little effect on the transport of ribose, thiocyanate and glutamate. Palmitic acid and palmitoyl-carnitine stimulate the entry of all the above metabolites. CoASH and carnitine have no effect on chloride uniport. Palmitoyl-CoA and the IMAC may have a role in controlling thermogenesis in liver mitochrondria.

Palmitoyl-CoA; Mitochondrial inner membrane; Thermogenesis; Inner membrane anion-conducting channel; Anion channel

1. INTRODUCTION

Palmitoyl-CoA has been shown to be involved in the control of various mitochondrial transport processes, such as the adenine nucleotide translocator [1], the transport of di- and tricarboxylic acids [1], and the uncoupling protein of brown fat mitochondria [2].

In brown fat mitochondria, in which facultative and adaptive heat production is a major function, palmitoyl-CoA and other long chain acyl-CoA derivatives stimulate anion-conduction by the thermogenin or uncoupling protein channel and have therefore been suggested to be in vivo activators of heat production [2]. Their mode of action is though to involve relief of the nucleotide-induced inhibition of anion(hydroxide)-conduction in brown fat mitochondria by competitive binding, but evidence for this action of palmitoyl-CoA having a physiological function is not as compelling as that for the free long chain fatty acids [3–5].

Now there is good evidence for a pH-dependent anion-conducting channel [6,7] in the inner membrane of rat liver mitochondria, the kinetics of which have been characterised in detail by Beavis and Garlid [8] who have given it the acronym IMAC (inner membrane anion channel). A similar, if not identical channel has been demonstrated in the inner membrane of cuprizone-induced giant mitochondria by Sorgato et al. [9] using patch clamping techniques. In this communication we present evidence that in rat liver mitochondria palmitoyl-CoA is a potent inhibitor of the pH-dependent IMAC, in contrast with free palmitic acid and palmitoyl-carnitine which stimulate anion uniport.

2. MATERIALS AND METHODS

2.1. *Materials*

Palmitoyl-CoA was purchased from BCL, Lewes, Sussex. Other acyl-CoA derivatives, palmitoyl-carnitine and carnitine were purchased from Sigma. All other chemicals were of analytical grade.

2.2. *Methods*

Mitochondria were prepared by the method of Selwyn et al.

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Abbreviations: CoASH, free coenzyme A; FCCP, carbonyl cyanide *p*-(trifluoromethoxy)phenylhydrazone; IMAC, mitochondrial inner membrane anion channel

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[10] from adult Wistar rats which had been starved overnight. The final suspension of mitochondria was between 70 and 90 mg protein/ml in 0.25 M sucrose medium containing 5 mM Hepes, pH 7.5. The protein concentration of the mitochondrial suspension was determined following the biuret method [11] and using BSA as standard. During any set of experiments the mitochondrial stock solution was stirred gently in air to ensure a constant control rate [12]. The standard assay for anion uniport was osmotic swelling (initiated by the addition of 12.5 μM FCCP to permit charge and pH balance of the chloride uniport) of mitochondria suspended in 0.1 M NH_4Cl containing 0.25 μg/ml each of rotenone and antimycin A, and 5 mM Hepes, adjusted to pH 8.0 with ammonia. Other media, when used, were all made up to 0.1 M (except ribose which was 0.25 M) and contained 5 mM Hepes, adjusted to pH 8.0 with either ammonia for ammonium salts or KOH for potassium salts and ribose. Mitochondrial swelling was measured by a decrease in light scattering [10].

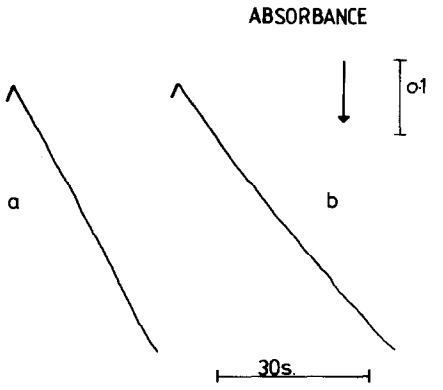

Fig.1. The effect of palmitoyl-CoA on mitochondrial swelling in NH_4Cl induced by FCCP. 4 mg protein were used in each run. (a) Without palmitoyl-CoA, (b) with 10 μM palmitoyl-CoA.

3. RESULTS

The recordings shown in fig.1 show the effect of 10 μM palmitoyl-CoA on mitochondrial swelling in NH_4Cl in the presence of the uncoupler FCCP. Palmitic acid, when added at this concentration has no detectable effect on anion conductivity, but when added at 60 μM a slight stimulation is observed (table 1). Fig.2 shows the dose-response curve for palmitoyl-CoA and as can be seen from this a maximal inhibition of 57% is observed at a palmitoyl-CoA concentration of 9.7 μM. Half-maximal inhibition is attained at a palmitoyl-CoA concentration of 2.4 μM. At concentrations greater than 10 μM no further inhibition is seen and this is probably due to a detergent action of palmitoyl-CoA damaging the membrane and causing increased permeability.

That the inhibitory effect of palmitoyl-CoA is not just due to the CoA moiety is indicated by the results obtained when CoASH (10 μM) is added. Only a 7% inhibition is observed. The importance of the chain length of the acyl group is shown in fig.3. As the chain length increases the inhibition of mitochondrial swelling increases to a maximum at palmitoyl-CoA. Stearoyl-CoA produces less inhibition than palmitoyl-CoA, which may be due to the stearoyl-CoA having a greater detergent type of effect than palmitoyl-CoA.

Table 1 shows the effect of other compounds related to palmitoyl-CoA on mitochondrial swelling in different media. Palmitoyl-carnitine and palmitic acid both stimulate mitochondrial swelling in all media used and it is likely that they have a detergent effect. This table also shows the specificity of palmitoyl-CoA for anion uniport via the IMAC since it produces no inhibition of ribose

Table 1

The effects of palmitic acid, palmitoyl-carnitine and palmitoyl-CoA on mitochondrial swelling in different media

	NH_4Cl	NH_4SCN	Ribose	K^+-glutamate	KCl
Palmitoyl-CoA (10 μM)	43	99	97	108	53
Palmitic acid (60 μM)	156	100	115	111	120
Palmitoyl-carnitine (60 μM)	300	135	152	127	237

Valinomycin (2 μg) was added to induce mitochondrial swelling in KCl; FCCP (12.5 μM) was added to induce mitochondrial swelling in NH_4Cl and NH_4SCN. Concentrations and pH of the media are as given in section 2. 4 mg mitochondrial protein were used in each run. Figures given are percentages of the absolute rate of swelling in each medium with no palmitic acid or derivative added

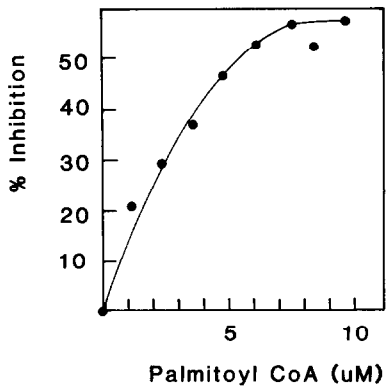

Fig.2. Inhibition of mitochondrial swelling in NH_4Cl by palmitoyl-CoA. 4 mg protein were used in each run.

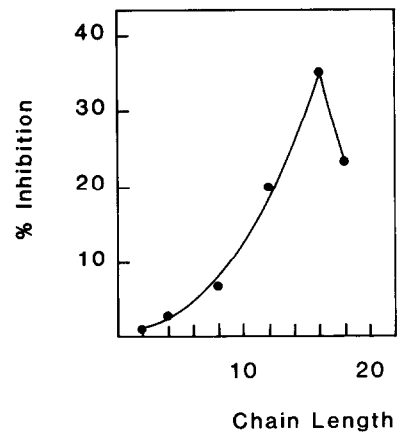

Fig.3. Showing the relationship between the chain length of acyl-CoA and inhibition of mitochondrial swelling in NH_4Cl. 4 mg protein were used in each run.

and glutamate entry, which both enter the mitochondria by carriers, or thiocyanate entry which is thought to diffuse across the lipid regions of the membrane. The similar inhibition of chloride transport in KCl with valinomycin and NH_4Cl with FCCP shows that it is the chloride entry that is inhibited rather than the cation or ionophore.

4. DISCUSSION

The low concentrations of palmitoyl-CoA required to inhibit the IMAC and the lack of effect of palmitic acid and CoASH indicate a palmitoyl-CoA-specific site in the components of this channel. These data are also further evidence that this channel is a discrete component of the mitochondrial inner membrane rather than an artefact or non-specific property.

The data also indicate that the operation of the channel in vivo may be controlled by palmitoyl-CoA which is known to be an allosteric effector of several mitochondrial processes including the adenine nucleotide translocator [1]. The inhibitory action of palmitoyl-CoA on the IMAC is the opposite of its stimulation of the anion conductivity of the brown fat mitochondrial uncoupling protein [2] which may reflect the differing functions of these mitochondria, heat production by brown fat mitochondria and energy conversion by liver mitochondria. In contrast to the effects on brown fat mitochondria in which both free fatty acids and palmitoyl-CoA stimulate anion-conduction [4] the present observations on liver mitochondria in which free fatty acids stimulate but palmitoyl-CoA inhibits are excellent evidence that the effect of palmitoyl-CoA is produced by specific binding rather than a detergent type of activity.

Sequence studies on the adenine nucleotide translocator, the phosphate carrier and the brown fat mitochondrial uncoupling protein [13–15], have shown extensive homologies and led to the suggestion that these proteins form an evolutionary related family. The property of allosteric control shared by the IMAC with the adenine nucleotide translocator and the brown fat mitochondrial uncoupling protein lends support to the idea that the IMAC is related to the brown fat mitochondrial uncoupling protein and hence to these other carriers. This inhibition by palmitoyl-CoA at physiological concentrations suggests a physiological role for the IMAC and palmitoyl-CoA in controlling the balance between thermogenesis and oxidative phosphorylation in liver mitochondria.

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