PALMITOYL COENZYME A: A POSSIBLE PHYSIOLOGICAL REGULATOR OF NUCLEOTIDE BINDING TO BROWN ADIPOSE TISSUE MITOCHONDRIA

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1. Introduction

The requirement for extramitochondrial purine nucleotides in order to obtain energy conservation in freshly isolated brown adipose tissue mitochondria is now well documented [1–3]. The specificity of the action correlates well with the effect of these nucleotides on inhibition of passive anion permeability in these mitochondria [4,5]. This inhibition has been demonstrated for chloride, bromide and nitrate but not for phosphate [4]. The site of action of the nucleotides has been shown to be outside the mitochondrial matrix [3,6] and thus, if the effect is of physiological importance, must be elicited by cytoplasmic nucleotides. Their action could be envisaged as inhibition of hydroxyl ion permeability. The concentrations of these nucleotides in vivo are well in excess of those required to accomplish energy conservation or inhibition of anion permeability in vitro [3,4,6]. It is therefore necessary to demonstrate the existence of a physiological antagonist to the nucleotide binding, which would allow a high effective proton conductance (alternatively hydroxide ion permeability) giving loose coupling and consequently thermogenesis, in spite of high cytoplasmic nucleotide concentrations.

As stated above, the nucleotide action on energy conservation correlates well with the inhibition of passive anion permeability. This ion permeability can be visualised as mitochondrial swelling in suitable media. It was therefore decided in this study to consider possible antagonists to the nucleotide action as being those agents which were able to reintroduce nucleotide-limited swelling. It is then possible to distinguish their action from those of regular uncouplers. This would not be possible in oxygen electrode experiments where respiratory control is taken as a measure of energy conservation [3].

Abbreviations: Palmitoyl CoA palmitoyl coenzyme A. CoASH, free coenzyme A. FCCP, carbonyl cyanide *p*-trifluoro methoxyphenylhydrazone. TES, *N*-tris (hydroxymethyl)methyl-2-aminoethane sulphonic acid.

2. Methods

Brown adipose tissue mitochondria were prepared from Syrian hamsters, cold-adapted for at least 3 weeks, by the method of Hittelman et al. for rat brown adipose tissue mitochondria [7]. The mitochondria were stored in 250 mM sucrose and mitochondrial protein was determined by the biuret method of Gornall et al. [8].

Mitochondrial swelling was measured as decrease in absorbance at 520 nm in a Hitachi-Perkin Elmer spectrophotometer No. 124. The temperature was 27°C and the volume in the cuvette was 2 ml. The mitochondrial protein concentration was 0.15–0.2 mg/ml. Other additions are shown in the legends to figures.

[^{3}H]GDP binding to mitochondria was determined by Millipore filtration in the presence of [^{14}C]sucrose according to Nicholls [6]. The mitochondrial protein concentration was 0.5 mg/ml.

Radiochemicals were from The Radiochemical Centre, Amersham, England, palmitoyl-L-carnitine was a gift from Otsuka Pharmaceutical Company, Osaka, Japan, FCCP from Pierce, Rockford, Illinios and all other chemicals of the highest purity commercially available.

3. Results and discussion

Thermogenesis in brown adipose tissue is caused by a high rate of fatty acid oxidation. It is thus likely that prior to the increased oxidation, the concentrations of the metabolic intermediates, long-chain acyl coenzyme A esters and long-chain acyl carnitine esters will increase in the mitochondrial intermembrane space and inner membrane. We have therefore tested the effect of these intermediates on nucleotide-induced inhibition of passive anion permeability and on nucleotide binding to mitochondria. GDP has been used as the test nucleotide.

Figure 1 shows the effect of GDP on passive swelling in the presence of potassium chloride plus valinomycin, and the effect of a subsequent addition of palmitoyl CoA. Valinomycin induces free permeability to K^+, so the effect of GDP – an inhibition of swelling – is due to a decreased Cl^- permeability [4]. Palmitoyl CoA reintroduces this swelling. It is also shown that an uncoupler is ineffective in this respect, since no proton movement is involved. Palmitate, when tested up to 20 μM, was also without effect. The same effect of palmitoyl CoA was demonstrated when potassium bromide or potassium nitrate were substituted for potassium chloride, while no effect was seen with potassium phosphate where a specific carrier is involved. This indicates that the effect of palmitoyl CoA is specific for the cases where GDP inhibits the anion permeability and is not, at these concentrations, an unspecific membrane detergent.

That palmitoyl CoA does not exert a detergent

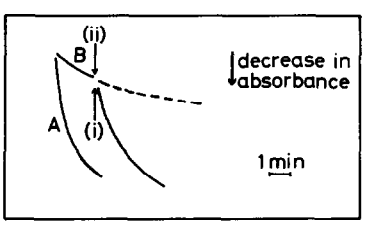

Fig.1. The effect of GDP, palmitoyl CoA and FCCP on passive swelling of brown adipose tissue mitochondria in potassium chloride. (A) Mitochondria 0.15 mg/ml in 2 ml 100 mM KCl, 5 mM K–TES, pH 7.2 + 0.5 μM valinomycin and 5 μM rotenone. The trace was started by the addition of mitochondria. (B) As A but with the further addition of 0.1 mM GDP initially. (i) at the point indicated 10 μM palmitoyl CoA was added. (ii) at the same point in a second experiment 0.5 μM FCCP was added.

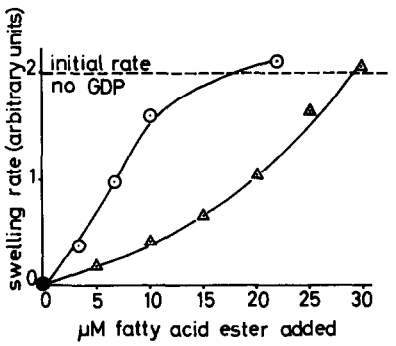

Fig.2. A comparison of the efficiencies of palmitoyl CoA and palmitoyl carnitine in inducing swelling in GDP-restricted mitochondria from brown adipose tissue. Medium as in fig.1. Mitochondria 0.2 mg/ml. GDP was present at 0.1 mM and the traces were started by the addition of mitochondria. (⊙——⊙) Palmitoyl CoA, (▲——▲) palmitoyl-L-carnitine.

effect at these concentrations is further indicated by the fact that the low rate of exogenous NADH oxidation seen in freshly isolated mitochondria is not stimulated by addition of palmitoyl CoA. Nor is palmitoyl CoA oxidised in the absence of added carnitine.

The intermembrane concentration of CoASH in hamster brown adipose tissue mitochondria is apparently high since further addition does not stimulate the rate of oxidation of fatty acids in the presence of ATP and carnitine (unpublished observations). This is in contrast to rat brown adipose tissue mitochondria [7]. Since this is the case, it is not surprising that even palmitoyl-L-carnitine is able to re-introduce swelling in GDP-inhibited mitochondria, as palmitoyl CoASH may be formed. This effect is shown in fig.2 and compared with palmitoyl CoA. Somewhat higher concentrations of palmitoyl carnitine are required.

In fig.3 it is demonstrated that the use of a high concentration of GDP is able to partially prevent the action of palmitoyl CoA; a 70–80% inhibition of the palmitoyl CoA effect is seen with 5 mM GDP. In an attempt to show that the effect of GDP here was related to its inhibitory action on anion permeability found in fresh mitochondria, the same experiment was carried out using 5 mM CDP. This pyrimidine nucleotide is relatively ineffective in inhibiting anion permeability in fresh mitochondria [5], and it was found to be without significant effect in preventing palmitoyl CoA-induced swelling. Indeed, the specificity of the

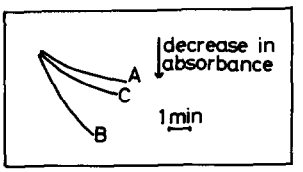

Fig.3. Demonstration of the competitive nature of the actions of GDP and palmitoyl CoA on passive swelling of brown adipose tissue mitochondria in potassium chloride. Medium as in fig.1. Mitochondria 0.15 mg/ml. All traces started by the addition of mitochondria. Trace A contains 0.1 mM GDP. Trace B contains 0.1 mM GDP and 5 μM palmitoyl CoA. Trace C contains 5 mM GDP and 5 μM palmitoyl CoA.

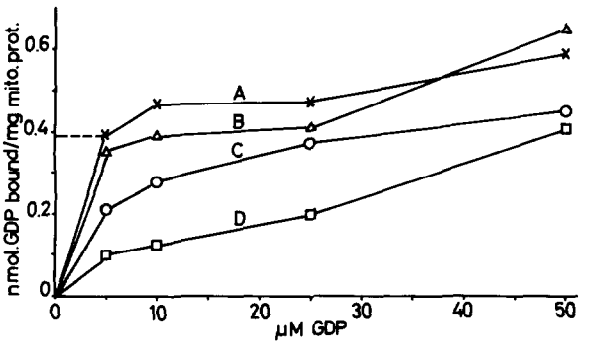

Fig.4. GDP binding to brown adipose tissue mitochondria. Binding determined according to Nicholls [6]. Mitochondria 0.5 mg/ml. (A) No further additions. (B) In the presence of 2 μM palmitoyl CoA. (C) In the presence of 5 μM palmitoyl CoA. (D) In the presence of 10 μM palmitoyl CoA.

prevention with different nucleotides followed closely that observed earlier for inhibition of passive Cl^- permeability [5]. In order to exclude that the action of GDP is related to its cation chelating ability, 5 mM EDTA was substituted for 5 mM GDP. No effect of EDTA was noted.

An additional indication that the effect of palmitoyl CoA reported here is distinct from its detergent effect was obtained by reintroducing swelling in nucleotide-limited mitochondria with various detergents. Lysolecithin, lubrol and deoxycholate were used at concentrations which gave the same degree of swelling as 5 μM palmitoyl CoA. 5 mM GDP failed to diminish this swelling.

Since coenzyme A possesses a terminal adenosine group which could be competitive with GDP, the influence of other adenosine-containing compounds on GDP-inhibited passive swelling was also tested. NAD^+, NADH, free coenzyme A, acetyl CoA, butyryl CoA and octanoyl CoA were completely ineffective when tested at concentrations up to 20 μM. Decanoyl CoA reintroduced about 15% of the original swelling rate at 20 μM. Since decanoyl CoA is also a strong detergent and yet relatively ineffective, this result is again an indication of the apparent specificity of the palmitoyl CoA effect. It seems possible that the long-chain acyl group is required to increase hydrophobicity.

GDP has been shown to bind to brown adipose tissue mitochondria [6,9]. We have therefore studied the influence of palmitoyl CoA on GDP binding at a variety of GDP and palmitoyl CoA concentrations (fig.4). It is evident that as the concentration of palmitoyl CoA is increased, higher concentrations of GDP are required to achieve the level of GDP binding observed at 5 μM GDP in the absence of palmitoyl CoA. The K_m of this binding is somewhat less than 5 μM [6]. An apparent K_i for palmitoyl CoA could be determined of 2.4 ± 1 μM (average of 3 experiments) at a mitochondrial protein concentration of 0.5 mg/ml. Since considerable unspecific binding of acyl CoA to proteins, lipids and natural membranes is known to occur, this figure would be very much lower if an extrapolation to infinite protein concentration was made [10,11]. These experiments were carried out with a 10 min incubation in order to obtain optimal GDP binding. However, it was shown in separate experiments that palmitoyl CoA prevented GDP binding within the time resolution of the experiment (< 1 min) i.e., GDP was not first bound and then removed unspecifically during slow membrane damage.

Experiments with isolated mitochondria designed to test the influence of GDP and albumin on induction of membrane potential have shown clearly that in the presence of albumin, much less GDP is required to achieve a given value of membrane potential, than in its absence [12]. This could be interpreted that albumin removes bound acyl CoA and therefore facilitates GDP binding. Flatmark and Pedersen [13] determined the acyl CoA concentration in isolated brown fat mitochondria from cold exposed guinea pig and reported 4.9 nmol/mg protein. This value is very much higher than in liver and close to the apparent K_i value calculated above. It is also timely to note that the effects described here should not be overlooked in

mitochondrial experiments where palmitoyl CoA and palmitoyl carnitine are supplied as substrates.

Long-chain acyl CoA esters have previously been shown to be inhibitory in a variety of enzyme reactions, e.g., adenine nucleotide translocase [14,15], nicotinamide nucleotide transhydrogenase [10], glycerol-3-phosphate dehydrogenase [16], the di- and tricarboxylate carriers [17] and a number of enzymes directly or indirectly coupled to fatty acid synthesis [18–20]. Their action has been suggested to be of physiological significance, although detergent effects have also been noted [21,22]. Pande and Blanchaer [14] using a ratio of palmitoyl CoA to mitochondrial protein similar to that used here, to study adenine nucleotide translocase, comment that the effect may be relevant for metabolic regulation in vivo and that at these concentrations intermembrane space enzymes, adenylate kinase and fatty acyl synthetase were not inhibited. Further, although the concentrations used here are in the range of the critical micelle concentration [23], it is difficult in the presence of membranes to evaluate the significance of this figure. Sumper and Träuble [11] showed that no free palmitoyl CoA was released from fatty acid synthetase but that albumin, dimyristoyllecithin dispersions or *E. coli* membranes were all extremely effective acceptors of palmitoyl CoA from this enzyme. Thus, the mitochondrial membranes can themselves function as efficient acceptors and reservoirs for palmitoyl CoA. It is felt that the results presented here demonstrate a possible physiological role of long-chain acyl coenzyme A esters in the induction of thermogenesis in brown adipose tissue. It may be envisaged that in the non-thermogenic state the nucleotide binding sites on the outer side of the inner mitochondrial membrane are saturated with cytoplasmic nucleotides. On initiation of thermogenesis, free fatty acids are formed from triglycerides by the action of hormone-sensitive lipase, these are activated in the outer mitochondrial membrane, and the acyl CoA formed may lead to a removal of the bound nucleotide and a consequent loosening of coupling of the mitochondria. A rapid rate of fatty acid oxidation is then possible. The mitochondria would then remain loosely coupled until lipolysis ceased, and the acyl CoA concentration diminished below the level antagonistic to nucleotide binding.

Acknowledgements

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