THE EFFECTS OF PALMITYL-COENZYME A AND ATRACTYLOSIDE ON THE STEADY-STATE INTRA- AND EXTRA-MITOCHONDRIAL PHOSPHORYLATION POTENTIALS GENERATED DURING ADP-CONTROLLED RESPIRATION

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1. Introduction

Long-chain fatty acyl-CoA esters have recently been shown in several laboratories to inhibit the mitochondrial adenine nucleotide translocase in a manner similar to that of atractyloside [1–6]. This work was apparently stimulated by the original report by Wojtczak and Zaluska [7] that translocation of adenine nucleotides is inhibited by oleic acid. It is now generally agreed, however, that coenzyme-A esters are much more effective inhibitors of the exchange than are free fatty acids and are considered by many workers to be natural effectors on the adenine nucleotide translocase.

It has been postulated that acyl-CoA mediated changes in the steady-state phosphorylation and reduction potentials in the cell, as well as effects on mitochondrial anion transport systems, may be important physiological controls, e.g. for diverting carbon in the liver to the synthesis of glucose and ketone bodies [2,5,6].

Detailed studies on the reversible inhibition of the adenine nucleotide translocase have been carried out primarily by measuring the initial rates of ATP–ADP or ATP–P_i exchange by mitochondrial suspensions incubated at low temperature in the presence of a respiratory inhibitor. In the present studies, experiments are reported on the marked effects of palmityl-CoA, as well as atractyloside, on the steady-state internal and external phosphorylation potentials generated by respiring liver mitochondria. The data are taken as additional support for the suggestion that long-chain acyl-CoA esters may play a direct role in the regulation of cellular metabolism at the level of the mitochondrial adenine nucleotide translocase.

2. Materials and methods

Rat liver mitochondria were prepared according to Johnson and Lardy [8] except that 1 mM EGTA (pH 7.4) was present in the isolation medium. Respiration of mitochondrial suspensions was stimulated to the desired rate by the addition of purified ATPase (F_1), exactly as reported previously [9.10]. Control titrations of ATPase were carried out in a Gilson 'oxygraph' immediately before carrying out the experiments. Methods for separation of mitochondria from the incubation medium, and for the determination of extramatrix water, internal adenine nucleotides and inorganic phosphate were all determined as reported [10]. Matrix water was taken to be 1.2 μl/mg of protein [11]. The concentrations of adenine nucleotides and inorganic phosphate in the extra-matrix space were determined from the total incubation mixture, since the size of the intramitochondrial pool was very small compared to the total.

3. Results and conclusions

Table 1 demonstrates the effects of increasing concentrations of palmityl-CoA or atractyloside on the steady-state intra- and extramitochondrial ATP/ADP ratios and the calculated phosphorylation potentials

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Table 1
The effects of palmityl-CoA and atractyloside on the phosphorylation potentials inside and outside the mitochondrial matrix

Inhibitor	(μM)	ATP/ADP	$[P_i]$ (mM)	ΔG_c (kcal/mol)	ATP/ADP	$[P_i]$ (mM)	ΔG_c (kcal/mol)	ΔG_c (out)	ΔG_c (in)
		(Outside)			(Matrix)				
Palmityl-CoA	0	43.7	2	5.9	3.8	13.8	3.3	−2.6	
	4	30.6	2	5.7	3.3	12.9	3.3	−2.4	
	8	23.2	2	5.5	4.3	12.0	3.4	−2.1	
	15	4.7	2	4.6	3.3	13.3	3.3	−1.3	
Atractyloside	0	38.8	2	5.8	3.7	13.8	3.3	−2.5	
1.5	1.5	7.8	2	4.9	4.5	15.1	3.4	−1.5	

Mitochondria (2 mg protein/ml) were incubated at 30°C in a medium containing 66 mM KCl, 33 mM Tris-HCl, 2 mM potassium phosphate, 2 mM metal ion-free ATP, 6 μCi 8-[^{14}C]ATP, 2 mM $MgCl_2$, 10 mM potassium-glutamate, 2 mM potassium-malate and palmityl-CoA or atractyloside as indicated to a final vol of 6 ml and a pH of 7.4. After a period of 1 min ATPase (determined from control incubations) required for obtaining 60% of State 3 [15] respiration (50% of maximum stimulation) was added. After an additional 5 min a 1 ml aliquot of the mixture was transferred to a centrifuge tube containing 0.3 ml cold 2 M $HClO_4$ for determination of total nucleotides and a 0.7 ml sample was transferred to a tube prepared with silicone oil layered over $HClO_4$ for determination of mitochondrial nucleotides and inorganic phosphate after separation form the medium. The mean values of duplicate determinations are reported.

when respiration is half-maximally stimulated by added ATPase. In the experiments reported elsewhere, it is shown that the above are truly steady-state values [10,12], and that it is very unlikely that the reported intramitochondrial ATP/ADP ratios are the result of an experimental artifact [12]. As shown in this table, the steady-state external ATP/ADP ratio maintained by the system when respiration is held constant is progressively decreased when increasing concentrations of palmityl-CoA are present. Atractyloside causes a similar effect, but at about 10-fold lower concentration. On the other hand, neither palmityl-CoA or atractyloside appreciably affects the internal ATP/ADP ratio. If it is assumed that the standard free energy of hydrolysis of ATP ($\Delta G_0'$) is the same within and without the mitochondrial matrix space, that the measured concentrations are equivalent to activities, and that the intramitochondrial nucleotides comprise a simple homogenous pool (see Klingenberg [13]), then the difference (out *minus* in) in the concentration terms (ΔG_c) represents the difference in the phosphorylation potentials (ΔGp) of the two compartments, where $\Delta Gp = \Delta G_0' + 1.36 \log [ATP]/[ADP][P_i]$. Thus, the difference in phosphorylation potentials out *minus* in, respiration is half-maximally stimulated by ADP (ATP_{ase}), is decreased from 2.6 to 1.3 kcal/mol as the concentration of palmityl-CoA is increased from 0 to 15 μM. Atractyloside has a similar effect. If this occurs in vivo, then an elevated concentration of acyl-CoA would be expected to effect a lowered ATP/ADP ratio in the cytosol and, through near-equilibrium reactions [14] in which both the adenine- and nicotinamide nucleotides are coenzymes, a decrease in the $NAD^+/NADH$ ratio.

If the data of table 1 are calculated to show the extramitochondrial ADP concentration required to give half-maximum stimulation of respiration, it is seen (table 2) that this value is increased from 43 to

Table 2
The effects of palmityl-CoA and atractyloside on the concentration of external ADP required for half-maximum stimulation of respiration. (Data are calculated from experiment shown in table 1)

Inhibitor	Concentration (μM)	[ADP] (μM)
Palmityl-CoA	0	43
	4	62
	8	81
	15	342
Atractyloside	0	49
	1.5	221

342 μM when the concentration of palmityl-CoA is increased from 0 to 15 μM, and to 221 μM ADP in the presence of 1.5 μM atractyloside. These values indicate a complex function rather than a purely competitive inhibition, owing in part to the fact that ATP is also an inhibitor competitive with ADP for entry into the mitochondria via the translocase, and that the external steady-state ATP/ADP ratio is altered in the presence of inhibitor (see e.g. [12]).

The data are taken to indicate that mitochondria determine in a primary manner the cytosolic phosphorylation potential (and indirectly the reduction potential through near-equilibrium coupled reactions), but that physiological effectors such as long-chain acyl-CoA can modify these potentials by directly affecting the movement of ADP into the mitochondria. If the total pool of intra-mitochondrial adenine nucleotides is indeed identical with the metabolically active pool, some doubt is raised from experiments as to the specific effects of ADP and ATP (or their ratio) on intra-mitochondrial enzymes.

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