EFFECT OF FATTY ACIDS AND ACYL-CoA ON THE PERMEABILITY OF MITOCHONDRIAL MEMBRANES TO MONOVALENT CATIONS

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1. Introduction

It is well known that monovalent metal cations can penetrate across the inner mitochondrial membrane only slowly [1–3] and that this penetration can be increased by several factors such as certain oligopeptide and macrolide antibiotics [3–6], certain nonionic detergents [7], chelating agents [8], SH group-blocking reagents [9,10], heavy metals [11–13] and acidic pH [14]. None of these factors occur, however, under normal conditions in the living cell. The present investigation shows that long chain fatty acids and their CoA thioesters at low concentration increase the permeability of mitochondria to monovalent alkali metal cations. This is manifested by both a passive outflow of intramitochondrial K^+ and an energy-dependent uptake of Li^+, Na^+, K^+, Rb^+ and Cs^+. This property of fatty acids and acyl-CoA may be of importance in controlling mitochondrial metabolism and configurational changes.

2. Materials and methods

Rat liver mitochondria were obtained by the conventional method [15] using 250 mM sucrose–2 mM Tris–HCl (or TEA–HCl), pH 7.4. Potassium was determined by atomic absorption, all other cations by flame photometry. Protein was measured by the biuret method [16] with bovine serum albumin as standard. Palmitoyl-CoA was either obtained from Sigma (St. Louis, Mo., USA) or synthesized as described by Seubert [17].

Abbreviations: TEA, triethanolamine; CCCP, carbonyl-cyanide-*m*-chlorophenylhydrazone.

3. Results

Mitochondria obtained in the present investigation contained, when freshly isolated, between 130 and 240 ng atoms potassium/mg protein. After 10 min incubation in isotonic buffered sucrose solution at 0°C about 30% of this potassium was released into the medium. This release was increased to 70% in the presence of CCCP (for uncoupler-induced potassium release from mitochondria see e.g. [14]). Oleate (fig. 1) and other long chain fatty acids also stimulated a release of K^+ under these conditions. At 100 nmoles oleate/mg mitochondrial protein the release was even larger than in the presence of the uncoupler CCCP. It is noteworthy that no substantial release of matrix proteins, as measured by malate dehydrogenase, occurred. It is also noteworthy that 50 nmoles oleate/mg mitochondrial protein is not uncoupling as shown by observations [18] that intramitochondrial energy-requiring pyruvate carboxylation is increased, and not decreased, under these conditions.

That oleate at concentrations used in fig. 1 was not uncoupling is also shown by the experiment in which an energy-dependent accumulation of potassium occurred when mitochondria were incubated in high

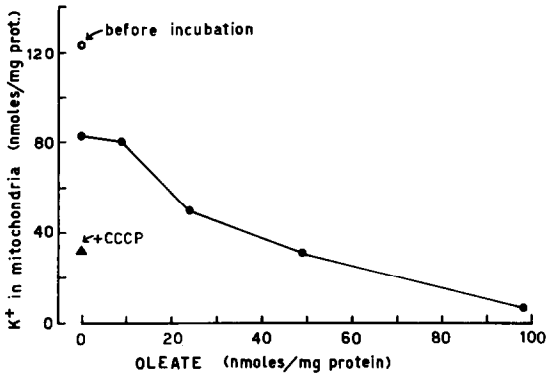

Fig. 1. Stimulation by oleate of the release of potassium from mitochondria. Mitochondria (15 mg protein) were incubated at 0°C in 3.0 ml of the medium containing 250 mM sucrose, 20 mM Tris–HCl (pH 7.1) and oleate as indicated. After 10 min the samples were centrifuged during 10 min at 15 000 *g* and K^+ was determined in the supernatants. Potassium remaining in mitochondria (●——●) was calculated as the difference between potassium content in mitochondria before incubation (○) and K^+ released into the supernatants. ▲ Indicates potassium remaining in mitochondria after incubation with 1 μM CCCP.

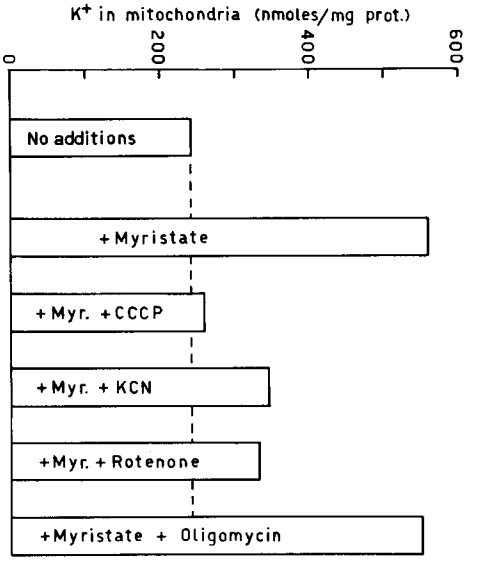

Fig. 2. Stimulation by myristate of potassium uptake by mitochondria. Mitochondria (7.5 mg protein) were incubated at 20°C in 2.0 ml of the medium containing 115 mM KCl, 15 mM sucrose, 19 mM Tris–HCl (pH 7.4) and 2.5 mM K acetate. The following additions were made where indicated: myristate (Myr.), 18 nmoles/mg mitochondrial protein; CCCP, 1 μM, KCN, 1.5 mM; rotenone, 2 μM; oligomycin, 1.3 μg/mg protein. After 10 min the samples were centrifuged at 0°C during 10 min at 15 000 *g*. The walls of the tubes and the pellets were gently and briefly rinsed with cold 250 mM sucrose, and the pellets were solubilized in 1.0 ml concentrated formic acid. After appriopriate dilution with water potassium was determined.

potassium medium. This is illustrated by fig. 2 where myristate was used instead of oleate. K^+ uptake stimulated by myristate was completely abolished by CCCP and strongly diminished by respiratory inhibitors potassium cyanide and rotenone. The effect of rotenone could be overcome if succinate was added as respiratory substrate (not shown). Oligomycin which does not collapse the energy state of mitochondrial membrane was without effect on potassium uptake. Oxidation of endogenous substrates was apparently a sufficient energy source for potassium accumulation since no further increase of K^+ uptake could be obtained upon succinate addition (not shown).

Fatty acids mimic in these experiments typical ionophores and, in fact, results identical to those of fig. 2 could be obtained using valinomycin instead of myristate. However, fatty acids differ from valinomycin by their lack of specificity towards monovalent alkali metal cations, as demonstrated in fig. 3. Valinomycin increased the accumulation of potassium, rubidium and cesium but not of lithium and sodium, which is in agreement with what is known about the specificity of this antibiotic [4]. Contrary to this, the accumulation of all five alkali metal cations was stimulated by myristate which resembles in this respect gramicidin [4,6].

Table 1 (expt. 1) shows that a substantial accumulation of K^+ occurred at very low amount of myristate. Further increasing the amount of the fatty acid had a relatively smaller effect on cation uptake. Omission of acetate from the medium diminished K^+ uptake. However, the dependence on acetate was less pronounced than is the case with the valinomycin-stimulated cation uptake [6]. Probably, the fatty acid itself may function as penetrating anion.

The effect of the fatty acid on cation accumulation is abolished by Mg^{2+} (table 1, expt. 2). This is analogous to the effect of divalent cations Mn^{2+} and Ca^{2+} on valinomycin-induced potassium uptake by mitochondria [19].

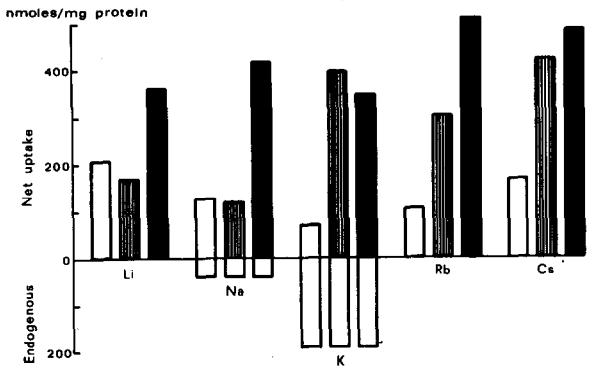

Fig. 3. Effect of myristate and valinomycin on monovalent cation uptake by mitochondria. The medium contained 100 mM metal chloride, 20 mM TEA–HCl (pH 7.2), 9 mM sucrose, 5 mM Tris acetate and 4.6 mg mitochondrial protein in total vol of 2.0 ml; valinomycin, 0.65 μg/mg protein, and myristate, 22 nmoles/mg protein, were also added where indicated. White columns, no additions; dashed columns, with valinomycin; black columns, with myristate. White columns below the zero line indicate the content of endogenous cations (Na^+ and K^+) in mitochondria (before incubation). Incubation, centrifugation and solubilization of the pellets for cation determination were carried out as described in the legend to fig. 2.

Among various fatty acids the highest stimulatory effect on K^+ accumulation in mitochondria has been repeatedly obtained with myristate (14 carbon atoms, saturated), laurate (12 carbon atoms, satur.) and oleate (18 carbon atoms, monounsaturated). Palmitate (16 C atoms, satur.) was slightly less active and octanoate (8 C, satur.) much less active. Very little effect was obtained with stearate (18 C, satur.) and none with caproate (6 C atoms, satur.). A potent stimulation of K^+ uptake was observed with palmitoyl-CoA (CoA esters of other fatty acids have not been studied). Energy-dependent potassium uptake in the presence of palmitoyl-CoA was usually higher than in the presence of same amounts of palmitate or myristate. However, carnitine strongly diminished the stimulation by palmitoyl-CoA, whereas it was without effect on stimulation by free fatty acids (table 1, expt. 3). Thus, the possibility was ruled out that the effect of fatty acids was due to the acyl-CoA formation.

In order to elucidate the mechanism by which fatty acids increase the permeability of mitochondrial membrane to monovalent cations their effect on the extract-

Table 1

Effect of fatty acids and palmitoyl-CoA on potassium uptake by mitochondria

Expt. No.	Additions	Potassium accumulated (ng atoms/mg protein)	
		Complete medium	Acetate omitted
1)	None	14	
	Myristate, 6.4 nmoles/mg protein	134	
	12.8 nmoles/mg protein	100	
	28 nmoles/mg protein	150	
	44 nmoles/mg protein	238	108
	87 nmoles/mg protein	231	
	170 nmoles/mg protein	314	
		Complete medium	+ 4.5 mM $MgCl_2$
2)	None	303	126
	Palmitate, 6.5 nmoles/mg protein	469	117
		Complete medium	+ 3.3 mM L-carnitine
3)	None	288	285
	Myristate, 12 nmoles/mg protein	465	557
	36 nmoles/mg protein	666	655
	Palmitate, 33 nmoles/mg protein	536	492
	Palmitoyl-CoA, 12 nmoles/mg protein	925	495

Incubation medium and the experimental procedure were as described in the legend to fig. 2. 'Potassium accumulated' designates the amount of potassium found in the pellet *minus* endogenous potassium in mitochondria before incubation (222, 137 and 145 ng atoms/mg protein in expts. 1, 2 and 3 respectively). No correction for extramitochondrial K^+ was made. Since spontaneous K^+ uptake (without any additions) differed considerably from one experiment to another, effects of additions and omissions are comparable within separate experiments only.

Table 2
Extraction of potassium by organic solvents in the presence of myristate

Composition of water phase	Myristate added (nmoles)	Potassium extracted into organic phase (ng atoms)
KCl 90 mM	0	0
	2500	2040
	5000	4680
KCl 90 mM + KOH 10 mM	0	120
	500	504
	1000	935
	2000	1950
KCl 90 mM + HCl 10 mM	2500	0
KCl 90 mM + Tris–HCl 10 mM (pH 7.4)	2500	0

Myristate was added to 10 ml of the water phase (saturated with isobutanol + benzene, 1:1 v/v) and the samples were extracted by vigorous shaking at room temperature with 5.0 ml of a mixture of isobutanol and benzene (1:1 v/v, saturated with water). Separation of the phases was facilitated by centrifugation, and aliquots of the organic phase were diluted with 5-fold volume of ethanol for potassium determination.

ability of patassium ions by organic solvents was studied. It was found (table 2) that upon addition of myristate to KCl solution a portion of potassium could be extracted in a mixture of isobutanol and benzene. The amount of extracted potassium was almost equivalent to the amount of added fatty acid. No extraction occurred from acidified solution or in the presence of Tris or TEA.

4. Discussion

It is shown in the present investigation that long chain fatty acids and palmitoyl-CoA greatly increase the permeability of mitochondrial membranes to monovalent alkali metal cations thus facilitating both passive outflow of mitochondrial potassium (in low potassium media) and energy-dependent cation uptake (at elevated external cation concentrations). Thus, the effect of fatty acids and palmitoyl-CoA resembles that of typical ionophore antibiotics. Similarly as for these antibiotics, two mechanisms of action of fatty acids and acyl-CoA on ion permeability can be discussed, namely that of a mobile carrier and the channel action. The carrier mechanism can be supposed for free fatty acids assuming that the penetrating species are undissociated salts of fatty acids with monovalent metals or cation–fatty acid complexes. This is supported by the observation (table 2) that fatty acids enable an almost stoichiometric extraction of potassium ions from water solution into organic solvents. On the other hand, channel mechanism seems more likely for palmitoyl-CoA since it is well known that acyl-CoA can not pass the inner mitochondrial membrane.

The ionophoric effect of fatty acids depends in essentially the same way on carbon atom chain length as the swelling effect [20,21]. This provides more evidence that the swelling effect of fatty acids on mitochondria [22–24] is due to increased cation permeability. Although there is a certain similarity to the dependence of the detergent effect of fatty acids on the chain length (compare fig. 2 in [21] and fig. 2 in [25]) it does not seem likely that the effect of fatty acids on cation permeation through mitochondrial membranes is entirely due to the detergent action. For example, laurate and myristate are almost equally effective in facilitating K^+ uptake (this investigation) and promoting swelling [21], while they differ in their detergent activity [25,26]. On the other hand, how-

ever, it has been observed that anionic detergents increase the permeability of bimolecular phospholipid films to cations [27].

The effect of fatty acids on cation uptake by mitochondria is abolished by Mg^{2+}. This may be due to the 'immobilizing' of fatty acids on mitochondrial membranes in the form of magnesium salts and/or to a specific effect of Mg^{2+} on permeability properties of mitochondrial membranes [8,19].

Increased membrane permeability to monovalent cations may partly explain the 'uncoupling' effect of fatty acids (for references see [28]). However, fatty acids differ in many respects from typical uncouplers (cf. [28]). At certain concentrations they even stimulate energy-dependent processes in mitochondria (e.g. [18]). Nevertheless, they increase energy dissipation, as illustrated for example in fig. 2 of [28]. This may be explained by a futile recycling of K^+ (energy-dependent uptake and passive outflow) as promoted by fatty acids.

It can also be supposed that small amounts of endogenous fatty acids and/or acyl-CoA in mitochondrial membranes are responsible for their normal low permeability to monovalent cations [1–3]. They are thus good candidates for the natural ionophore which can be functioning during energy-dependent volume and configurational changes of mitochondria both in vitro [29–34] and in situ [30,33,35]. The unusually high permeability to monovalent cations observed in brown adipose tissue mitochondria [36] is most likely also due to their high content of fatty acids [37,38].

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