THE INHIBITION OF TRANSLOCATION OF ADENINE NUCLEOTIDES THROUGH MITOCHONDRIAL MEMBRANES BY OLEATE

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Fatty acids are well known uncoupling agents of oxidative phosphorylation (Pressman and Lardy, 1956; Scholefield, 1956; Lehninger and Remmert, 1959; Borst et al., 1962). The uncoupling effect depends on the length of the carbon atom chain and is also influenced by the presence of unsaturated linkages, better uncouplers being acids with medium chain length of 12 or 14 carbon atoms and those containing unsaturated bonds. However, fatty acids differ in some respect from typical uncouplers, like 2,4-dinitrophenol (DNP). The most striking phenomenon is that, in the presence of Mg^{2+} low concentrations of uncoupling fatty acids inhibit the DNP-stimulated mitochondrial ATPase, and only higher concentrations are stimulatory[x]. This effect, first observed by Bos and Emmelot (1962), is also shown in Fig. 1. The minimum ATPase activity has usually been obtained with 25 to 50 mumoles oleate/mg mitochondrial protein. The same amount of oleate completely uncouples oxidative phosphorylation and maximally stimulates the controlled respiration of mitochondria. The latent, Mg^{2+}-

[x] In the absence of added Mg^{2+}, or in the presence of excess EDTA, oleate strongly inhibits mitochondrial ATPase at low, as well as at high, concentrations (Borst et al., 1962; Bos and Emmelot, 1962). In this case the picture is probably complicated by removal of intramitochondrial magnesium, as will be discussed elsewhere (Wojtczak and Zaluska, in preparation).

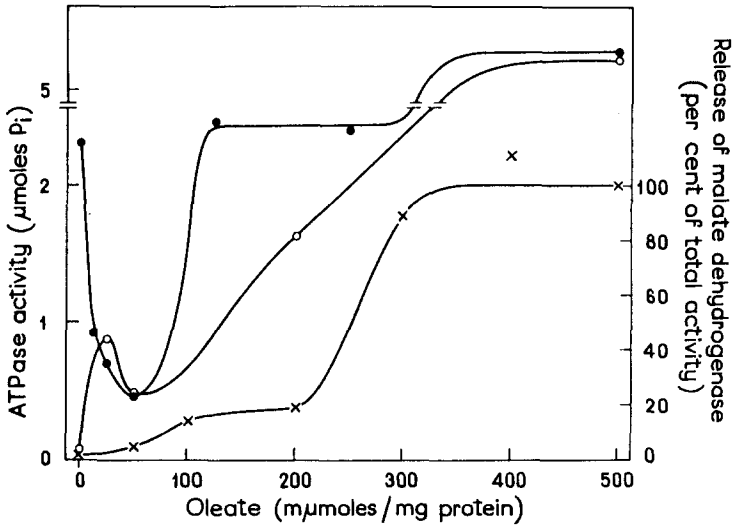

Fig. 1. Effect of oleate on mitochondrial DNP-stimulated (●——●) and latent (o——o) ATPases and on the release of malate dehydrogenase (x——x). Incubation mixture for ATPase: KCl 75 mM; Tris-Cl (pH 7.4) 40 mM; $MgCl_2$ 1 mM; DNP (only for ●——●) 0.1 mM; ATP 3.3 mM or 6.7 mM (at higher oleate concentrations); and 2 mg mitochondrial protein; total volume 1.5 ml; temperature 20°; incubation time 15 min. Incubation mixture for the release of malate dehydrogenase was the same except that ATP and DNP were omitted and the amount of mitochondria was increased to 6 mg protein. For the determination of malate dehydrogenase the incubation mixture was centrifuged to remove mitochondria and the clear supernatant was analysed for enzyme activity according to Bergmeyer and Bernt (1963). Activity of the dehydrogenase was expressed as percentage of the total activity released during sonication of mitochondria.

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activated, ATPase of mitochondria is stimulated by oleate; however, even in this case a minimum activity at 50 mμmoles oleate/mg protein can also be occasionally observed (Fig. 1; cf also Bos and Emmelot, 1962, Fig. 8). The inhibition by oleate of the DNP-stimulated ATPase was difficult to explain on the basis of the known effects of fatty acids on mitochondria. The present paper shows that oleate, and probably other uncoupling fatty acids, are inhibitors of the translocation of adenine nucleotides through mitochondrial membranes.

Rat liver mitochondria were incubated at 0° with ^{14}C-labelled ATP for periods of time between 30 sec. and 2 min., following which the

translocation of adenine nucleotides was stopped by atractyloside (Klingenberg and Pfaff, 1966; Vignais and Duée, 1966), the incubation mixture diluted with an equal volume of 0.25 M sucrose, and mitochondria separated by centrifugation. After one or two washings with 0.25 M sucrose, they were solubilized in deoxycholate solution and total radioactivity of the mitochondria was measured. It corresponded to ATP (and other adenine nucleotides) which entered into the mitochondria during the incubation. The results, illustrated in Fig. 2, show that the uptake of ^{14}C-ATP by mitochondria is linear with time within at least 2 min. The extrapolation of the radioactivity to zero time reveals an atractyloside-insensitive exchange of adenine nucleotides, while the slope of the line represents the rate of an atractyloside-sensitive process (cf Klingenberg and Pfaff, 1966; Vignais and Duee, 1966). It can be seen that oleate produces a drastic

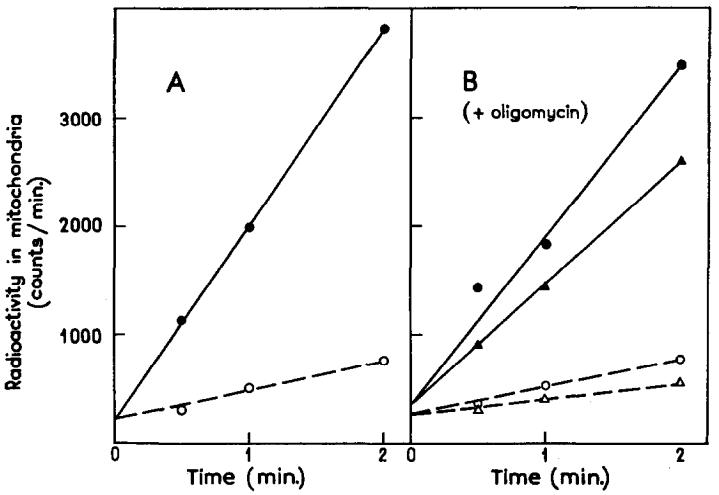

Fig. 2. Effect of oleate on the translocation of ^{14}C-ATP through mitochondrial membranes. Incubation mixture: KCl 120 mM; Tris-Cl (pH 7.4) 20 mM; $MgCl_2$ 1.1 mM; sucrose 25 mM; ATP 60 µM (20,000 counts/min. ^{14}C); and 7.0 mg (A) or 13.4 mg (B) mitochondrial protein; the mixture in B also contained 15 µg oligomycin. Total volume 2.2 ml; temperature 0° (ice-bath). ●——●, control; o- - -o, 25 mµmoles oleate/mg protein; ▲——▲, 0.1 mM DNP; Δ- - -Δ, 0.1 mM DNP + 25 mµmoles oleate/mg protein. The incubation was stopped by the addition of 60 mµmoles (A) or 90 mµmoles (B) atractyloside.

decrease of this latter rate, while it has little or no effect on the atractyloside-insensitive transport. The mean value for the inhibition of the atractyloside-sensitive translocation of ATP in the presence of 25 mμmoles oleate/mg protein amounts to 87% (4 experiments). The inhibition is not dependent on the stimulation by oleate of the latent ATPase, since it can also be observed in the presence of oligomycin; DNP has only a negligible effect on the translocation (Fig. 2 B; cf also Klingenberg and Pfaff, 1966).

The rate of the translocation of ADP was of the same order of magnitude as that of ATP and was inhibited 72% (one experiment) in the presence of 25 mμmoles oleate/mg protein. The translocation of AMP appeared to be much slower (which is compatible with the observations of Klingenberg and Pfaff, 1966) and was also inhibited by oleate.

It is concluded that oleate, apart from its uncoupling effect on oxidative phosphorylation, exerts a potent inhibitory effect on the translocation of adenine nucleotides through mitochondrial membranes, thus inhibiting the DNP-stimulated mitochondrial ATPase. This inhibition, however, can be observed only in the presence of low concentrations of oleate which are not sufficient to cause major structural alterations of mitochondria. Oleate is a known swelling agent for mitochondria (Lehninger and Remmert, 1959). However, during the present investigation, indications of large-scale swelling, as measured photometrically, were observed only with amounts of oleate exceeding 50 mμmoles/mg mitochondrial protein. Higher amounts of oleate produced damage in the mitochondrial structure, resulting in a "leakage" of intramitochondrial proteins (Drahota, personal communication). Fig. 1 shows the parallelism between release of mitochondrial malate dehydrogenase and reactivation of the DNP-stimulated ATPase in the presence of higher amounts of oleate. It is suggested that in swollen (or disrupted) mitochondria the oleate-sensitive barrier no longer exists and ATP has free access to the ATPase site. Compatible with this is

the observation that, at higher concentrations of oleate, mitochondrial ATPase is no longer sensitive to atractyloside (Table I). Still higher concentrations of oleate (0.5 μmole/mg protein) probably induce a lysis and disintegration of mitochondria, accompanied by a further stimulation of the ATPase well above the level obtained with DNP, and by a release of total mitochondrial malate dehydrogenase (Fig. 1).

TABLE I

Effect of atractyloside on DNP-stimulated mitochondrial ATPase in the presence of oleate.
(Conditions as in Fig. 1; atractyloside 7.5 mμmoles).

Oleate (mμmoles/mg protein)	P_i liberated (μmoles)	
	without atractyloside	with
0	2.5	0.6
75	0.9	0.8
500	4.8	5.0

It is reasonable to assume that the inhibition of DNP-stimulated ATPase by other long-chain fatty acids (Chefurka and Dumas, 1966; Załuska and Wojtczak, unpublished) has a similar explanation. It also seems likely that the inhibition of mitochondrial ATPase by low concentrations of palmitoylcarnitine, as recently observed by Dargel and Strack (1967), is due to a similar inhibition of ATP translocation.

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