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Inhibition by oleate of the binding and exchange of ATP by mitochondrial membranes

It has been demonstrated previously[1,2] that oleate and, to a lesser extent other long-chain fatty acids inhibit the translocation of adenine nucleotides through mitochondrial membranes, thus apparently simulating the known effect of atractyloside[3–6]. The inhibition by oleate of ATP translocation was found[2] to be correlated with the initiation of mitochondrial swelling, as observed in electron microscope. Recently, WINKLER AND LEHNINGER[7] have shown that a membrane preparation obtained from mitochondria by solubilizing them with a nonionic detergent, Lubrol, has the ability to bind ATP and ADP and to exchange them with the nucleotides of the medium. As both the binding and the exchange are inhibited by atractyloside, the authors suggest that they reflect the property of the intact membrane to translocate adenine nucleotides. It was therefore interesting to examine whether the binding and the exchange of adenine nucleotides by the membrane preparation are susceptible to fatty acids similarly as is the translocation of these nucleotides in intact membranes[1,2].

The membrane fraction was obtained from rat liver mitochondria by solubilizing them with Lubrol (I.C.I. Organics, Providence, R.I., U.S.A.) exactly as described by WINKLER AND LEHNINGER[7]. The incubation of these membranes with [^{14}C]ATP also followed the procedure described by these authors.

Table I shows the effect of oleate on the binding of [^{14}C]ATP by the membrane fraction. About 200 nmoles oleate per mg membrane protein were usually required to inhibit completely the atractyloside-sensitive binding. This is several times more than the amount of oleate giving maximum inhibition of nucleotide translocation in intact liver mitochondria[1,2], but the latter inhibition was never as strong as that produced by atractyloside.

TABLE I

EFFECT OF OLEATE ON THE BINDING OF [^{14}C]ATP BY MITOCHONDRIAL MEMBRANE PREPARATION

Incubation medium: 250 mM sucrose, 10 mM Tris–HCl (pH 7.4), 1 mM EDTA, 7 μM [^{14}C]ATP containing 14800 counts/min per nmole, and 2.9 mg membrane protein; total volume, 6.0 ml. After incubation for 20 min at 0° the mixture was centrifuged at 125000 × *g* during 60 min to separate the membranes.

Additions to the medium	[^{14}C]*ATP bound to the membranes*	
	Counts/min	*Relative values*
None	1670	100
Oleate, 191 nmoles/mg protein	1100	66
Atractyloside, 17 μM	900	54

Similar to atractyloside[7], oleate did not produce a release of adenine nucleotides already bound to the membranes, but it inhibited the exchange of bound ATP or ADP with external adenine nucleotides. This is shown in Table II where 250 nmoles oleate per mg protein give the same result as 10 μM atractyloside.

TABLE II

EFFECT OF OLEATE ON THE EXCHANGE OF BOUND [^{14}C]ATP WITH EXTERNAL UNLABELED ATP

The membranes were preloaded with [^{14}C]ATP as described by WINKLER AND LEHNINGER[7], and samples corresponding to 3.56 mg protein were then incubated in the medium containing 250 mM sucrose, 10 mM Tris–HCl (pH 7.4), 1 mM EDTA and 48 μM unlabeled ATP; total volume was 6.0 ml. After 20 min at 0° the membranes were separated by centrifugation and the radioactivity remaining therein was determined.

Additions	[^{14}C]*ATP remaining bound to the membranes (counts/min)*
None	2490
Oleate, 50 nmoles/mg protein	2700
Oleate, 100 nmoles/mg protein	3140
Oleate, 250 nmoles/mg protein	3600
Atractyloside, 10 μM	3590
None, unlabeled ATP omitted	6180

These results allow one to speculate that oleate and, most likely, other long-chain fatty acids produce some conformational alteration of that entity of mitochondrial membrane which is involved in the translocation of adenine nucleotides. A similar conformational change has been postulated by WINKLER AND LEHNINGER[7] to explain the effect of atractyloside.

In summary, oleate inhibits the binding of ATP by a membrane preparation of liver mitochondria obtained by using the nonionic detergent, Lubrol. When acting upon membranes already containing bound ATP, oleate inhibits its exchange with external adenine nucleotides. In both respects, the effect of oleate simulates that of atractyloside.

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