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The protecting effect of L-carnitine on Ca^{2+}-loaded rat liver mitochondria

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It is shown that L-carnitine strongly increases the ability of rat liver mitochondria to respond to the train of Ca^{2+} additions by a transient stimulation of the State-4 respiration rate. Such an effect requires ATP and the L-carnitine efficiency strongly decreases when ATP is omitted. Oleate influences the mitochondria in a fashion opposite to that of L-carnitine. The oleate effect is strongly diminished by L-carnitine. Again, the L-carnitine effect requires ATP, and D-carnitine fails to substitute for L-carnitine. It is suggested that L-carnitine removes, in an ATP-dependent manner, endogenous or added fatty acids, which are involved in oxidative damage of Ca^{2+}-loaded mitochondria.

Mitochondrion; Fatty acid; Ca^{2+}; Carnitine; Acyl-CoA; Uncoupler

1. INTRODUCTION

Although as long ago as in 1961 Wojtczak and Lehninger found that fatty acids increase Ca^{2+}-dependent swelling of mitochondria [1], the precise role of fatty acids in this process is still debated. This swelling was explained by the Ca^{2+}-dependent activation of phospholipase A_2 and the main role was attributed to lysophospholipids [2]. Hunter and Haworth found that simultaneous addition of Ca^{2+} and oleate in micromolar concentrations not only causes swelling but also uncoupling of the oxidative phosphorylation due to a non-specific increase in the permeability of inner mitochondrial membrane [3].

At present, the Ca^{2+}-dependent permeabilization of the inner mitochondrial membrane is considered to be the result of either the formation of cyclosporin A-sensitive pores or/and of defects in the membrane lipid phase caused by phospholipase A_2 reaction products ([4,5] and references therein).

Recently Pfeiffer and co-workers published data suggesting that Ca^{2+}-dependent mitochondrial damage is due to fatty acyl-CoA derivatives [6]. The experiments with low concentrations of fatty acids added to mitochondria and other results seemed to suggest that fatty acids do not play an essential role in mitochondrial permeability transition [6]. However, the local concentration of fatty acids produced by phospholipase A_2 may be significantly higher than their total concentration; moreover, their interaction with other components should be considered.

In the present study, an attempt to get a further insight into the role of fatty acids in Ca^{2+}-dependent damage of mitochondria was made. Instead of albumin, which non-specifically binds fatty acids, lysophospholipids, etc., L-carnitine was used, which enzymatically transforms fatty acids and fatty acyl-CoA to acyl carnitine.

It should be stressed that Ca^{2+}-dependent mitochondrial damage is considered to have a physiological function [5], and the possible protective effect of carnitine may be interesting in a medical perspective.

Abbreviations: FCCP, *p*-trifluoromethoxycarbonylcyanide phenylhydrazone; DNP, 2,4-dinitrophenol, MOPS, morpholinopropane sulphonate; BSA, bovine serum albumin

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2. MATERIALS AND METHODS

Male rats (Sprague–Dawley strain, approx. 200–350 g), fasted for 24 h before the experiment, were used. Liver mitochondria were isolated, using the solutions described in [7], the supernatant of 10% homogenate being centrifuged at 850 × *g* for 10 min; mitochondria were sedimented at 14 500 × *g* for 10 min, resuspended and centrifuged at 8000 × *g* for 10 min. The mitochondria were suspended in medium containing 250 mM sucrose, 5 mM MOPS, 0.1 mM EGTA, BSA (0.2 mg/ml); the suspension contained 60–80 mg protein per ml.

Respiration was recorded by a Clark-type oxygen electrode and Biological Oxygen Monitor YSI (Terzano, Italy) at 30°C. $\Delta\psi$ was recorded using a Ph_4P^+-sensitive electrode at 30°C. The incubation medium contained 250 mM sucrose, 5 mM MOPS, 3 mM P_i buffer, 5 mM succinate, pH 7.4. Oligomycin (1 μg/ml), 1 mM ATP and carnitine were added before mitochondria.

The protein concentration was measured by the biuret method. The

concentration of mitochondrial protein was about 1 mg/ml.

Fatty acid-free BSA, Ruthenium red, MOPS, oleate, oligomycin, ATP, EGTA (Sigma), FCCP (Fluka), succinate (Boehringer), L- and D-carnitine (Sigma-Tau) were used. All reagents were of pure, analytical grade.

3. RESULTS AND DISCUSSION

Fig. 1 shows that the first three additions of 40 μM $CaCl_2$ result in a reversible stimulation of the State-4 respiration of rat liver mitochondria. After the fourth addition, the respiration rate steadily increases; an irreversible inhibition develops which cannot be released by a further addition of $CaCl_2$ or DNP. This indicates that the fourth Ca^{2+} addition causes the permeabilization of the inner mitochondrial membrane [8].

To estimate the Ca^{2+} retention, we took into account the sum of $CaCl_2$ additions which preceded that, inducing the steady increase in respiration rate.

0.5 mM and 5 mM L-carnitine strongly increase Ca^{2+} retention in mitochondria (Fig. 1, Table I). 0.5 mM D-carnitine is ineffective (Table I). These results were confirmed when $\Delta\psi$ was measured with the Ph_4P^+-sensitive electrode (data not shown).

Omission of ATP from the incubation medium exerts a little (if any) effect on Ca^{2+} retention in samples without L-carnitine but practically abolishes the effect of L-carnitine (Table I).

5 μM oleate and 10 nM FCCP (which exert a partial and approximately equal stimulation of the respiratory rate) decrease the Ca^{2+} retention to about the same degree; the data suggest that the decrease of Ca^{2+} retention is connected with the induction of H^+ conductance by oleic acid.

Table I

Effect of carnitine on the Ca^{2+} retention by rat liver mitochondria

Additions	Ca^{2+} retention (μM Ca^{2+})
—	149 ± 38
5 mM L-carnitine	160 ± 33**
ATP	171 ± 45
0.5 mM L-carnitine + ATP	320 ± 80*
5 mM L-carnitine + ATP	335 ± 78*
0.5 mM D-carnitine + ATP	160 ± 40
oleate	113 ± 64
oleate + 5 mM L-carnitine	120 ± 40**
oleate + ATP	90 ± 40
oleate + 0.5 mM L-carnitine + ATP	259 ± 30*
oleate + 5 mM L-carnitine + ATP	250 + 37*
oleate + 0.5 mM D-carnitine + ATP	116 ± 26

*$P < 0.001$ with respect to the same sample but without L-carnitine, **$P < 0.01$ with respect to the same sample but without ATP. Additions, 1 mM ATP, 10 μM oleate. Other conditions as in Fig. 1. Right column, the sum of $CaCl_2$ additions (in μM) preceded that, inducing the steady increase in respiration rate. The data represent mean ± SD of 3–9 experiments.

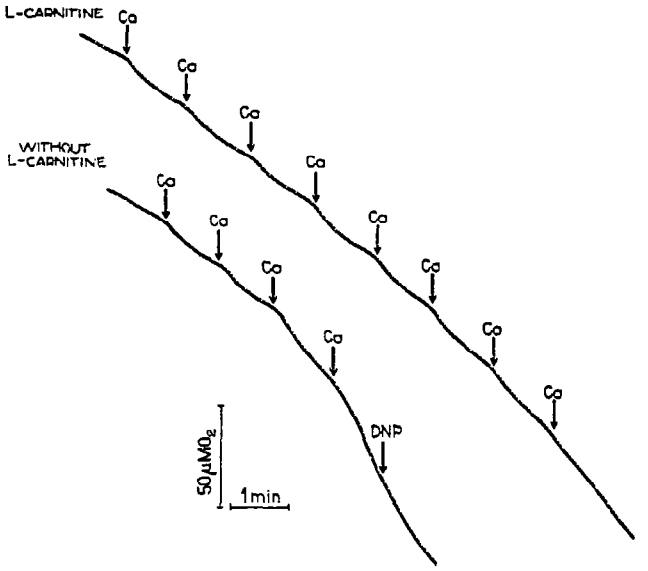

Fig. 1. The effect of L-carnitine on oxygen consumption during a train of $CaCl_2$ additions to rat liver mitochondria. The incubation medium contained 250 mM sucrose, 1 mM $MgCl_2$, 5 mM MOPS, oligomycin (1 μg/ml), 1 mM ATP, 3 mM P_i buffer, pH 7.4, 5 mM succinate; mitochondria, 1 mg protein × ml^{-1}. Additions: 40 μM $CaCl_2$ (Ca). (5 mM L-carnitine was added before mitochondria (upper curve).

As can also be seen in Table I, 10 μM oleate decreases about twice the Ca^{2+} retention and L-carnitine relieves this damage, whereas D-carnitine has no effect. Omission of ATP from incubation medium does not decrease the Ca^{2+} retention. Again the protective effect of L-carnitine is observed only in the presence of ATP (Table I).

It is well established that L-carnitine can increase the Ca^{2+} retention in the presence of added acyl-CoA by converting acyl-CoA to acyl carnitine; the same carnitine effect without added acyl-CoA was explained by the involvement of endogenous fatty acyl-CoA [9].

One may expect that L-carnitine can also exert recoupling effect by decreasing the concentrations of endogenous fatty acids, since fatty acids may be converted to acyl-CoA and then to acyl carnitine. High concentrations of acyl-carnitine were demonstrated to decrease energy coupling and Ca^{2+} retention but this effect was much smaller than that of acyl-CoA and fatty acids [10,11].

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