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Anion permeation limits the uncoupling activity of fatty acids in mitochondria

Peter Schönfeld

Institut für Biochemie der Medizinischen Akademie Magdeburg, O-3090 Magdeburg, Germany

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The action of such membrane-permeant cations as tetraphenyl phosphonium and dibenzyldimethyl ammonium upon fatty acid-uncoupled respiration has been studied with oligomycin-inhibited rat liver mitochondria. Both cations enhance fatty acid-stimulated respiration. This synergistic effect is explained by a facilitated permeation of the fatty acid anion across the inner membrane due to an ion-pair complex. It is concluded that fatty acid uncoupling in rat liver mitochondria is limited by fatty acid anion permeation.

Anion permeation; Fatty acid uncoupling activity; Respiration; Mitochondria

1. INTRODUCTION

To explain the mechanism of the well-known uncoupling effect of long-chain fatty acids on mitochondrial oxidative phosphorylation, Rottenberg and co-workers introduced the decoupling concept some years ago [1,2], namely that fatty acids are decouplers stimulating electron transport in the respiratory chain without decreasing the electrochemical proton gradient. In recent studies, however, new experimental facts concerning the uncoupling effect of fatty acids were gained. First it was found that the ATP/ADP antiporter is partly involved in the stimulation of mitochondrial respiration by long chain fatty acids (LCFAs) [3–5]. Secondly, LCFAs increase the conductance of membranes to protons like the artificial protonophores, DNP or FCCP [4,6–10]. Within this view is the discussion of whether the protonated as well as the deprotonated fatty acid is able to transfer to the inner membrane [7,8,10]. As there is no effective charge delocalization in the fatty acid anion it is reasonable to assume that the back-permeation of the anion across the lipophilic phase of the inner membrane is the rate-limiting step in such a proton–carrier mechanism.

To find out about such a rate-limiting step the influence of membrane-permeant cations on the uncoupling activity of fatty acids was investigated in the present study. From the finding that membrane-permeant cations, such as dibenzyldimethyl ammonium and tetraphenyl phosphonium, enhance the stimulation of respiration by LCFA, it is concluded that the LCFA-mediated H^+ conductance of the inner membrane is in line with an A^- carrier transport mechanism [7].

Abbreviations: LCFA, long-chain fatty acids; RLM, rat liver mitochondria; DNP, 2,4-dinitrophenol; FCCP, *p*-trifluoromethoxycarbonylcyanide phenylhydrazone; DDA^+, *N,N*-dibenzyl *N,N*-dimethyl ammonium cation; TPP^+, tetraphenyl phosphonium cation; $TPMP^+$, triphenylmethyl phosphonium cation; TBA^+, tetrabutyl ammonium cation.

Correspondence address: P. Schönfeld, Institut für Biochemie, Medizinische Akademie Magdeburg, Leipziger Str. 44, O-3090 Magdeburg, Germany.

2. MATERIALS AND METHODS

Mitochondria were isolated from the liver of female albino rats (150–200 g body weight) by a standard procedure [11]. The protein content in the stock suspension was determined by the biuret method [12]. Functional integrity was determined by measuring the respiratory control ratio with ADP (0.4 mM) and with glutamate plus malate as respiratory substrates (5 mM/5 mM). Only mitochondria showing respiratory control ratios greater than 5 were used. All incubations were done at 25°C, and oxygen uptake by mitochondria was measured as in [8]. The incubation medium was the same as in [8], but supplemented with the F_0F_1-ATPase inhibitor, oligomycin (2 μg/ml), throughout.

Tris, tricine, FCCP and ADP were from Boehringer, TPP^+ and DDA^+ were from Ferak and fatty acids were purchased from Merck.

3. RESULTS

Fig. 1 shows how the membrane-permeant cations, TPP^+ and DDA^+, affect basal as well as palmitate-stimulated respiration of rat liver mitochondria. It can be seen that palmitate-stimulated respiration increases proportionally with the added concentration of cations while their effect on basal respiration is negligible. That TPP^+, which is known to be more lipophilic than DDA^+, exerted a stronger enhancement on respiration indicates the importance of cation lipophilicity to developing this synergistic effect. Both membrane-permeant

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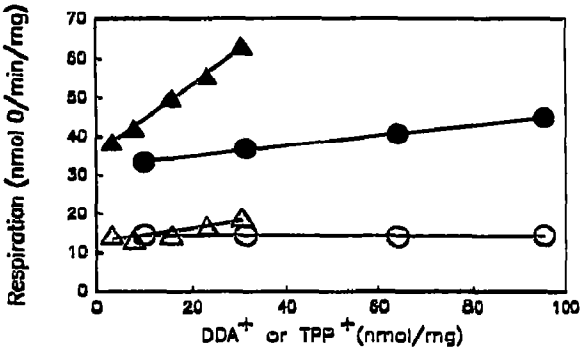

Fig. 1. Effect of lipophilic cations on palmitate-uncoupled respiration. TPP^+ (▲,△) or DDA^+ (●,○) were added to the incubation mixture in the absence (△,○) and presence (▲,●) of 16 μM palmitate. The mitochondrial protein content was 0.67 mg/ml.

cations, however, did not induce an uncoupling activity in the presence of fatty acid esters like 1-palmitoylcarnitine (not shown).

An enhancement of respiration by DDA^+ and TPP^+ was not only seen with palmitate but also with fatty acids of a shorter chain length. As Fig. 2 shows, however, the ability of TPP^+ to enhance respiration declined as the uncoupling potency of the fatty acids decreased. This observation suggests that lipophilicity of fatty acids is also crucial to the development of the synergistic effect.

In a previous study with rat liver mitochondria we demonstrated that there is a striking similarity in the characteristics of the uncoupling effect of LCFAs and the protonophoric effect of artificial protonophores [8]. Thus we studied the effect of TPP^+ on the stimulation of respiration by DNP and FCCP. As Fig. 3 shows, TPP^+ increases the sensitivity of rat liver mitochondria to the uncoupling activity of DNP (synergistic effect 52%) while that of FCCP was increased only slightly (synergistic effect 18%). To explain the enhancement of the protonophore-based H^+ conductivity of the inner

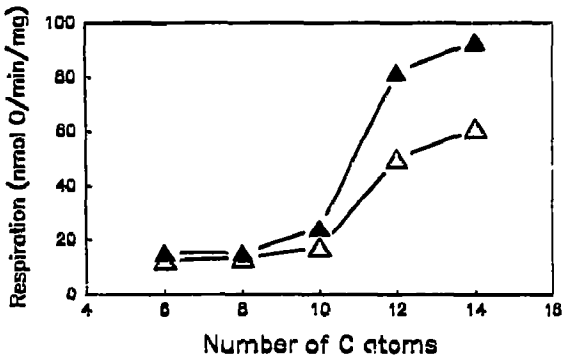

Fig. 2. Stimulation of respiration by fatty acids of varying chain length without and with TPP^+. The concentration of the fatty acids was approximately 93 μM, and that of TPP^+ 31 μM. The mitochondrial protein content was 0.85 mg/ml.

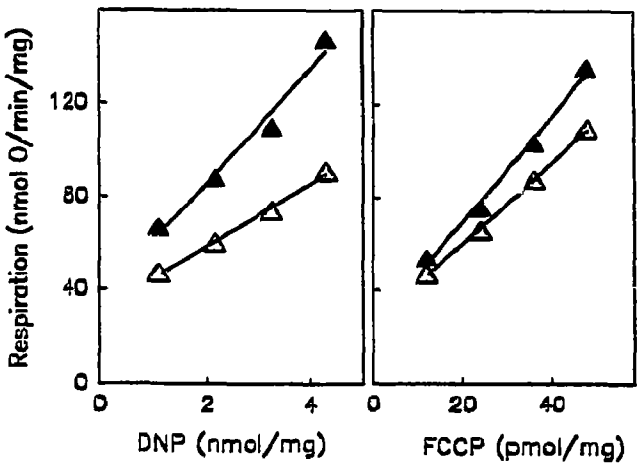

Fig. 3. Effect of TPP^+ on DNP- and FCCP-stimulated respiration. Respiration was partially uncoupled by DNP and FCCP in the absence (△) and presence (▲) of 48 μM TPP^+. The mitochondrial protein content was 1.35 mg/ml.

membrane by lipophilic cations the formation of an ion-pair complex between the anionic form of the protonophore and the cation was recently postulated [13]. From that, the pronounced synergistic effect of TPP^+ on DNP-stimulated respiration is explained by a stronger electrostatic interaction with its anionic form. In the case of FCCP, the negative charge is more effectively delocalized in its anionic form, resulting in a weaker tendency to form an ion-pair complex with TPP^+.

4. DISCUSSION

This study shows that in incubations of rat liver mitochondria with fatty acids plus lipophilic cations respiration is stimulated in a non-additive manner (Fig. 1). A

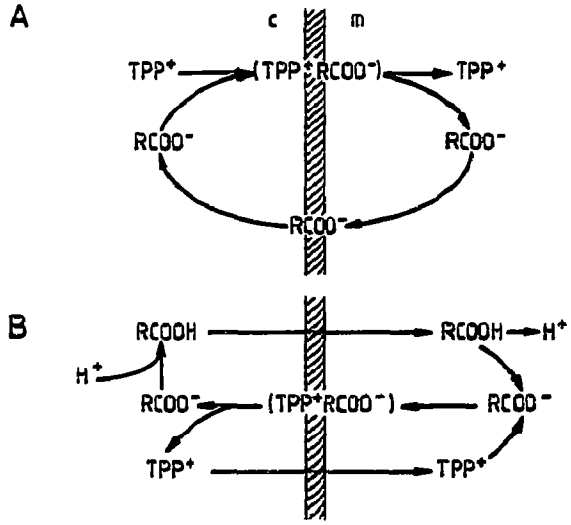

Fig. 4. Two models of a facilitated charge transport through the inner membrane based on ion-pair formation between the fatty acid anion and the lipophilic TPP^+ cation. For explanation see text.

similar observation was reported from studies where rat liver mitochondria were incubated with the membrane-permeant tetraphenyl boron anion plus lipophilic cations, such as DDA^+, $TPMP^+$ and TBA^+ [14]. In the latter case the mechanism of respiration stimulation could be explained by the TPB^--mediated facilitation of an electrophoretic uptake of lipophilic cations, resulting in a decrease of the electrochemical proton gradient. If such a mechanism for the non-additive stimulation of respiration found with fatty acids plus DDA^+ or TPP^+ is adopted then the fatty acid anion is thought to permeate the inner membrane freely (see Fig. 4A). Back-permeation of the fatty acid in its undissociated form would not result in a decrease of the electrochemical proton gradient since then the uptake of a positive charge is balanced by the transport of a proton from the matrix side to the extramitochondrial side. For this reason it is suggestive to explain the non-additive stimulation of mitochondrial respiration within the framework of a membranal fatty acid circuit as the result of lipophilic cation-facilitated permeation of the fatty acid anion across the inner membrane (Fig. 4B). Hence, the missing uncoupling activity of short-chain fatty acids can be understood from the inability of their anions to permeate the membrane bulk phase, a property which is connected with a poor anion lipophilicity. In accordance with this explanation for the synergistic effects of TPP^+ and DDA^+ on fatty acid-stimulated respiration is the earlier suggestion that fatty acids act as simple (A^--type) proton carriers in phospholipid bilayer membranes [7].

The results obtained in this study demonstrate that a positively charged mobile molecule incorporated in the membrane is able to enhance the uncoupling activity of fatty acids. This view is in line with the hypothesis postulated recently that the ATP/ADP antiporter, which is generally believed to have positive charges at the receptor site, acts as translocation carrier for the fatty acid anion in fatty acid uncoupling (for review see [4,15]).

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