Available online at www.sciencedirect.com
ScienceDirect
Biochimica et Biophysica Acta 1757 (2006) 1324–1329
BIOCHIMICA ET BIOPHYSICA ACTA
BBA
www.elsevier.com/locate/bbabio

Participation of ATP/ADP antiporter in oleate- and oleate hydroperoxide-induced uncoupling suppressed by GDP and carboxyatractylate

Lyudmila S. Khailova [a], Elena A. Prikhodko [c], Vera I. Dedukhova [a], Elena N. Mokhova [a], Vasily N. Popov [b], Vladimir P. Skulachev [a,c,*]

[a] *Department of Bioenergetics, A. N. Belozersky Institute of Physico-Chemical Biology, Moscow State University, Moscow 119992, Russia*
[b] *Department of Plant Physiology and Biochemistry, Voronezh State University, Voronezh 394693, Russia*
[c] *Faculty of Bioengineering and Bioinformatics, Moscow State University, Moscow 119992, Russia*

Received 13 February 2006; received in revised form 10 April 2006; accepted 28 April 2006
Available online 9 May 2006

Abstract

In experiments on isolated kidney and liver mitochondria, it is shown that oleate hydroperoxide induces a much smaller increase in the controlled respiration rate and $\Delta\Psi$ decrease than the same concentrations of oleate. Palmitate appears to be less efficient than oleate but more efficient than oleate hydroperoxide. In all cases, GDP and CAtr cause some recoupling, CAtr being more effective. Addition of 0.2 mM GDP before CAtr does not prevent further $\Delta\Psi$ increase by subsequent CAtr addition. On the other hand, GDP added after CAtr is without any effect. GDP partially prevents the $\Delta\Psi$ lowering by ADP at the State 4 — State 3 transition if small amounts of CAtr are present. The data are consistent with the suggestion of F. Goglia and V.P. Skulachev (*FASEB J.* 17, 1585–1591, 2003) that fatty acid anions are translocated by mitochondrial anion carriers much better than their hydroperoxides. As to GDP recoupling, it cannot be regarded as a specific probe for uncoupling by UCPs since it can be mediated by the ATP/ADP antiporter.

Keywords: Mitochondria; Uncoupling; Fatty acid; Peroxide; Uncoupling protein; ATP/ADP antiporter

1. Introduction

The fatty acid-induced uncoupling in mitochondria is known to be mediated by some anion carriers (for review, see [1]). For most of these carriers, e.g., for the ATP/ADP antiporter, this uncoupling looks like a function additional to the major one, i.e., translocation of anionic substrates such as ATP and ADP. On the other hand, an uncoupling resulting in heat generation via fatty acid-mediated increase in the inner membrane proton conductance seems to be the only function of UCP1 in brown fat mitochondria. According to the fatty acid cycle hypothesis, UCP1 and the ATP/ADP antiporter participate in an uncoupling process by facilitating the efflux of fatty acid anions through hydrophobic barrier of the inner mitochondrial membrane [2].

It is well established that proton conductance induced by UCP1 in the presence of fatty acids is suppressed by GDP, GTP, ADP, and ATP (for reviews, see [3,4]). Similar properties are inherent in a number of minor uncoupling proteins (e.g., UCP2 and 3) discovered during recent years (for reviews, see [1,5]). It should be emphasized that the protonophorous effect of these new UCPs and their regulation have been studied mainly in experiments on UCP-containing proteoliposomes ([6–8] and Refs. within). Investigations of minor UCPs in mitochondria are very difficult since, in contrast to UCP1, their concentrations in the mitochondrial membrane are extremely low.

Usually, uncoupling effect of UCPs is identified by its inhibition with GDP (see, e.g., [9,10]). On the other hand, a possible contribution of such anion carriers as the ATP/ADP antiporter should be taken into account when the mechanism of the GDP recoupling is studied. In recent studies on the recoupling activity

Abbreviations: BSA, bovine serum albumin; CAtr, carboxyatractylate; $\Delta\Psi$, transmembrane electric potential difference; DNP, 2,4-dinitrophenol; LOOHs, products of peroxidation of phospholipids or fatty acids; ROS, reactive oxygen species; UCP, uncoupling protein

* Corresponding author. Department of Bioenergetics, A. N. Belozersky Institute of Physico-Chemical Biology, Moscow State University, Moscow 119992, Russia. Tel.: +7 95 9395530; fax: +7 95 939 0338.
E-mail address: skulach@belozersky.msu.ru (V.P. Skulachev).

0005-2728/$ - see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbabio.2006.04.024

of GDP, a pronounced recoupling effect of carboxyatractylate (CAtr), a highly specific and powerful inhibitor of the ATP/ADP antiporter, was also found [10–12].

The ATP/ADP antiporter is involved in the fatty acid-induced uncoupling in all studied mitochondria [for review, see [1]] if the incubation medium pH is not too low (for liver mitochondria, higher than 7.0 [13]). The ADP/ATP antiporter is very specific not only to transporting but also to binding of nucleotides, so guanine nucleotides bind to the antiporter with much lower affinity than ADP [14]. On the face of it, this fact argues against the involvement of the ATP/ADP antiporter in the GDP-induced recoupling of the fatty acid-uncoupled mitochondria. However, it remains to be studied whether this is true for the antiporter in the presence of LOOHs that were shown to change some properties of the ATP/ADP antiporter (see Refs. in [15–17]). Moreover, recoupling by GDP is studied at 0.5–1 mM concentrations of the nucleotide. This is much higher than the binding constant for ADP of the ATP/ADP antiporter.

In several recent publications, hypotheses concerning interactions of the minor UCPs with LOOHs have been presented. Brand and colleagues suggested that LOOHs activate UCPs [9,10] and that the main role of reactive oxygen species (ROS) consists here in induction of lipid peroxidation [9,10,16]. According to a hypothesis of Goglia and Skulachev [18], UCPs, but not the ATP/ADP antiporter, can transport fatty acid hydroperoxide anions from matrix to intermembrane space. The authors proposed also that fatty acid hydroperoxides in their neutral form cross the inner mitochondrial membrane with a much lower rate compared with fatty acids due to the presence of the –OOH group [18]. Later, however, Jezek, Garlid, and colleagues found that linoleic acid hydroperoxide, like non-oxidized linoleic acid, causes a fast flip-flop-dependent acidification of liposomes. Their experimental data with UCP2-containing proteoliposomes suggest that this hydroperoxide increases proton conductance of the proteoliposomal membrane [8].

In the present study, we have found that (i) oleic acid hydroperoxides are much weaker uncouplers than oleic acid in kidney and liver mitochondria, (ii) GDP and CAtr recouple mitochondria uncoupled by oleate, (iii) CAtr being added before GDP completely prevents the GDP-induced recoupling, and (iv) GDP partially prevents the Ψ-lowering effect of ADP at the State 4 — State 3 transition if the ATP/ADP antiporter becomes the rate-limiting step due to addition of small amount of CAtr. The first observation is in line with Goglia and Skulachev's suggestion [18] that the ATP/ADP antiporter fails to carry out fast export of anions of fatty acid hydroperoxide. Observations (ii)–(iv) indicate that it is the ATP/ADP antiporter rather than UCP that is target for the recoupling effect of GDP in kidney mitochondria. Apparently, the ATP/ADP antiporter cannot transport GDP but can bind GDP in a fashion partially inhibiting transport of ADP as well as of fatty acids, provided that the GDP concentration is sufficiently high.

2. Materials and methods

Mitochondria were isolated by differential centrifugation from kidneys or livers of white rats weighing about 200 g. Liver mitochondria were isolated as described earlier [13]. For isolation of kidney mitochondria, the homogenization medium contained 250 mM sucrose, BSA (0.5 mg/ml), 2 mM EGTA, 5 mM MOPS–KOH, pH 7.4. After homogenization (the tissue/isolation medium ratio was about 1:8), homogenate was centrifuged at 700×*g* for 5 min, mitochondria were sedimented at 8000×*g* for 10 min, resuspended in 1 ml of the isolation medium of the same composition as the homogenization medium but supplemented with higher BSA (3 mg/ml), diluted with 30 ml medium containing 0.5 mg BSA per ml, and centrifuged at 200×*g* for 5 min. The supernatant was centrifuged at 12,000×*g* for 10 min. The mitochondrial pellet was resuspended in about 200 μl of isolation medium. Protein concentration in the final mitochondrial suspension was 90–120 mg/ml. Mitochondrial protein content was measured using the Biuret method.

ΔΨ was estimated using the safranin O dye [19]. The difference in the absorbance between at 555 and 523 nm (ΔA) was recorded with an Aminco DW-2000 spectrophotometer in the dual wavelength mode.

The following incubation media were used. (1) Salt incubation medium, 120 mM KCl, 10 μM safranin O, 2 μM rotenone, BSA (0.1 mg/ml), 1 mM EGTA, 5 mM sodium succinate, 5 mM potassium phosphate, 3 mM HEPES–KOH (pH 7.2). (2) Sucrose medium, 250 mM sucrose, BSA (0.1 mg/ml), a respiratory substrate, 1 mM EGTA, 100 μM potassium phosphate, 5 mM MOPS–KOH (pH 7.4). The mitochondrial protein content was 0.5–0.6 mg protein/ml. The temperature was 26 °C.

MOPS, palmitic acid, oleic acid, oligomycin, carboxyatractylate, fatty acid-free BSA, GDP, rotenone, HEPES, and succinate were from Sigma; EGTA, safranin O, potassium phosphate, and pyruvate were from Serva; DNP was from Merck; FCCP was from Fluka; KCl was from AnalaR. Sucrose was twice precipitated with bidistilled ethanol from a concentrated solution in bidistilled water. Oleic acid stock solution in bidistilled ethanol (30 mM) was stored at −10 °C and diluted to 5 mM oleic acid before experiment.

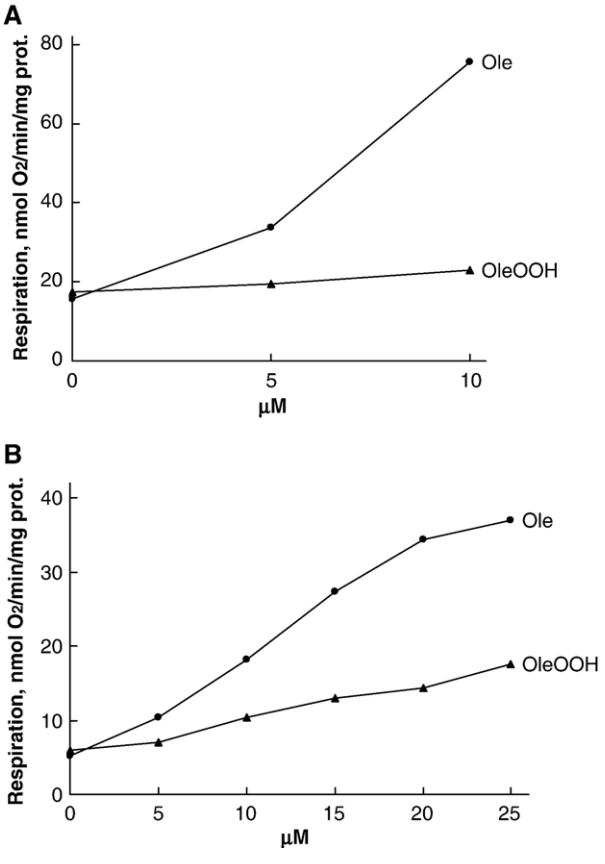

Fig. 1. Concentration dependence of oleic acid (Ole)- and oleic acid hydroperoxide (OleOOH)-induced effects on couple respiration (A, B). In panel A, the incubation medium contained 250 mM sucrose, 2×10^{-6} M rotenone, BSA (0.1 mg/ml), oligomycin (2 μg/mg protein), 1 mM EGTA, 5 mM succinate, 5 mM KH_2PO_4, 5 mM MOPS–KOH (pH 7.4) and kidney mitochondria (0.8 mg protein/ml). In panel B, liver mitochondria (1.2 mg protein/ml), incubation medium as in A, except concentration of KH_2PO_4 was decreased to 0.1 mM.