Volume 264, number 2, 246–248 FEBS 08442 May 1990

Does the function of adenine nucleotide translocase in fatty acid uncoupling depend on the type of mitochondria?

Peter Schönfeld

Institut für Biochemie der Medizinischen Akademie Magdeburg, Magdeburg, GDR

Received 27 March 1990

The stimulation of respiration by long-chain fatty acids and FCCP was studied with oligomycin-inhibited mitochondria from rat liver, heart and kidney tissue. By addition of equal amounts of palmitate and oleate, mitochondrial respiration was increased in the order RLM < RKM < RHM. Using the classical protonophore FCCP, this difference could not be observed. Inhibition of oleate-stimulated respiration by carboxyatractyloside decreased in the order RHM > RKM > RLM. As CAT sensitivity of oleate-stimulated respiration and the mitochondrial ANT content were found to be correlated, it is suggested that the weak CAT sensitivity of oleate-stimulated respiration of RLM [(1989) Biochim. Biophys. Acta 977, 266–272] is due to the low content of ANT.

Uncoupling; Fatty acid; Mitochondria; Adenine nucleotide translocase

1. INTRODUCTION

Free long-chain fatty acids (LCFA) have been known as uncouplers of oxidative phosphorylation for many years (for review see [1]). The mechanism, however, which does the uncoupling is still under discussion [2–6]. Recently, a new insight into their uncoupling effect was gained. First, it was found that LCFA induce a H^+ cycling through the mitochondrial inner membrane as known from artificial protonophores [3,5,6]. Second, the stimulation of mitochondrial respiration by LCFA is decreased by CAT and other inhibitors of the adenine nucleotide translocase [4,5]. Because of these findings and the fact that LCFA have, compared with artificial protonophores, only a weak potency to increase the H^+ conductance of protein-free bilayer membranes [5,7,8], an involvement of ANT in the uncoupling process by fatty acids was postulated [4,5]. A mechanism which may explain this involvement could hence be the back-transport of deprotonated LCFA from the matrix to the extramitochondrial space by ANT [4,5]. Due to its low charge-delocalization in the molecule, the deprotonated fatty acid is considered as a poor permeant in the phospholipid region of biomembranes [8].

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

Abbreviations: LCFA, long-chain fatty acids; ANT, adenine nucleotide translocase; CAT, carboxyatractyloside; FCCP, *p*-trifluoromethoxycarbonylcyanide phenylhydrazone; RLM, rat liver mitochondria; RHM, rat heart mitochondria; RKM, rat kidney mitochondria

In studies with rat liver mitochondria, we saw that CAT has only a small inhibitory effect on respiration stimulated by oleate [6]. That led us to the conclusion that in this type of mitochondria the adenine nucleotide translocase is involved in the uncoupling effect of LCFA just to a minor extent. With mitochondria from rat skeletal muscle, however, a stronger effect of CAT on respiration has been demonstrated [4]. The question arose as to what the reason is for the dependence of the CAT-sensitivity of LCFA-stimulated respiration on the kind of mitochondria.

Based on results obtained with mitochondria from rat liver, heart and kidney tissue, it is concluded in this study that the sensitivity of LCFA-uncoupled respiration to CAT depends on the content of ANT in mitochondria.

2. MATERIALS AND METHODS

Mitochondria were isolated from the liver [9], heart [10] and kidney cortex [11] of female albino rats (150–200 g body weight) by standard procedures. The protein content of the mitochondrial stock suspension was measured by a biuret method [12]. Functional integrity was determined by measuring the respiratory control ratio with ADP (0.4 mM) and glutamate plus malate (5 mM + 5 mM) as substrates. Only mitochondria showing respiratory control ratios greater than 5 were used.

The incubation medium contained in mM: sucrose 90, tricine 60, $MgCl_2$ 5, Na_2-EDTA 0.5, glucose 15, nicotinamide 40, sodium phosphate 10; adjusted to pH 7.4 by HCl and gassed by air. Oxygen uptake by mitochondria was measured polarographically recording the oxygen concentration trace and its first derivative ($d[O_2]/dt$). The incubations were performed at 25°C in a closed, thermostated and magnetically stirred vessel equipped with a Clark-type electrode. The ANT content was estimated from titration curves obtained by stepwise addition of CAT to isolated mitochondria respiring at the active state.

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/90/$3.50

Tris, tricine, succinic acid, FCCP, ATP, ADP and yeast hexokinase (EC 2.7.1.1) were obtained from Boehringer; CAT and oligomycin from Sigma. Oleate and palmitate was purchased from Merck and used as 5 mM solution in ethanol.

3. RESULTS AND DISCUSSION

Fig. 1 shows the effect of CAT on the respiration of mitochondria from heart, kidney cortex and liver being incubated at different oleate concentrations. The values of mitochondrial respiration were obtained from separate incubations where an excessive amount of CAT was added after oleate. The mitochondria were preincubated with oligomycin to prevent an activation of added fatty acids within the matrix space. As respiratory substrates glutamate plus malate were used.

The inhibitory effect of CAT on the oleate-stimulated respiration is seen to be quite different in these mitochondria. CAT decreased the oleate-stimulated respiration of RHM much more than that of RLM. Similar results were produced with palmitate (not shown). These demonstrate that the effect of CAT on the LCFA-stimulated respiration depends on the type of mitochondria.

It is known that mitochondria from heart and liver vary in their ANT contents [13]. The differing inhibitory effect of CAT found with heart and liver could therefore be caused by the differing ANT content. To check this, the ANT contents of RHM, RLM and RKM were determined and compared with the sensitivity of oleate-stimulated respiration to CAT. From earlier studies it is known that CAT reacts with ANT in a 1:1 stoichiometry [14,15]. That is why the ANT content in mitochondria may be estimated by titration of their active-state respiration with CAT as illustrated in Fig. 2. For quantifying the CAT sensitivity we adopted the following equation:

$$\text{CAT sensitivity} = \left(1 - \frac{\text{slope } (+\text{CAT})}{\text{slope } (-\text{CAT})}\right) \times 100$$

The data compiled in Table I show that there is a wide range of ANT contents with mitochondria from different sources. Data also show clearly that the CAT sensitivity of oleate-stimulated respiration rises with a rising mitochondrial ANT content. Another aspect is the variable responding behaviour of mitochondria to changes in the oleate concentration. As an example, the slope of the respiration/oleate relationship of RHM is twice that of RLM. The dependence of the slope on the type of mitochondria is a specific finding for the LCFA-stimulated respiration and was not found with the respiration uncoupled by the artificial protonophore FCCP (Fig. 3). For the latter, no effect of CAT on FCCP-stimulated respiration is reported [5] which excludes an involvement of ANT in the uncoupling by this protonophore. It may be concluded that an increase in the ANT content results in an increased response of mitochondria to uncoupling by LCFA. This finding provides further support for an involvement of ANT in uncoupling by fatty acids. The higher sensitivity of ANT-rich mitochondria to fatty acid uncoupling substantiates the old idea of Skulachev [16] that fatty acids act as endogeneous uncouplers for heat generation in skeletal muscle tissue of cold-exposed animals.

Lastly, the slope of CAT-insensitive respiration seems to be independent of the kind of mitochondria (Table I) although they vary remarkably in their inner

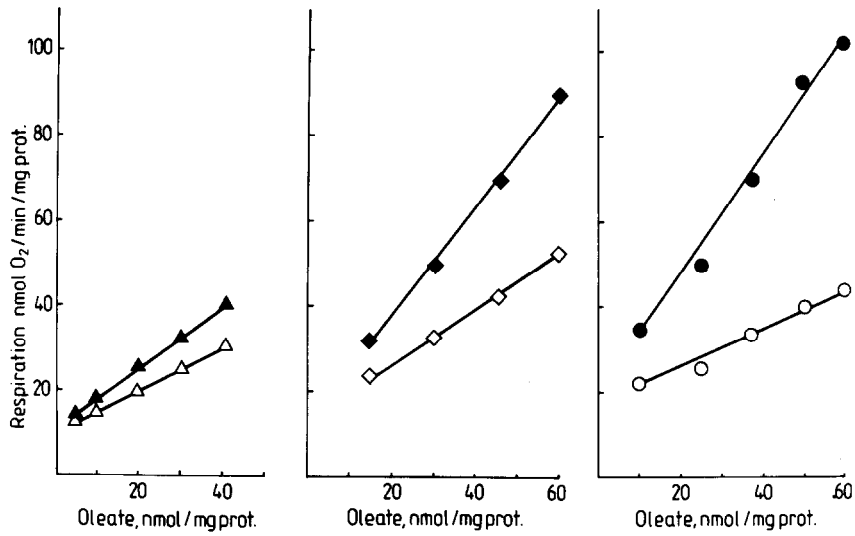

Fig. 1. The effect of CAT on the oleate-stimulated respiration of mitochondria from rat heart, liver and kidney cortex. RHM (0.65 mg/ml), RLM (1.6 mg/ml) and RKM (1.2 mg/ml) were added to the incubation medium which had been supplemented with oligomycin (2 μg/ml). After 3 min oleate was added. As soon as the oleate-stimulated respiration was stationary, CAT (3 μmol/ml) was added. The following symbols were used in all figures: (○,●) RHM; (△, ▲) RLM; (◇, ◆) RKM; (●, ▲, ◆) without CAT; (○, △, ◇) with CAT.

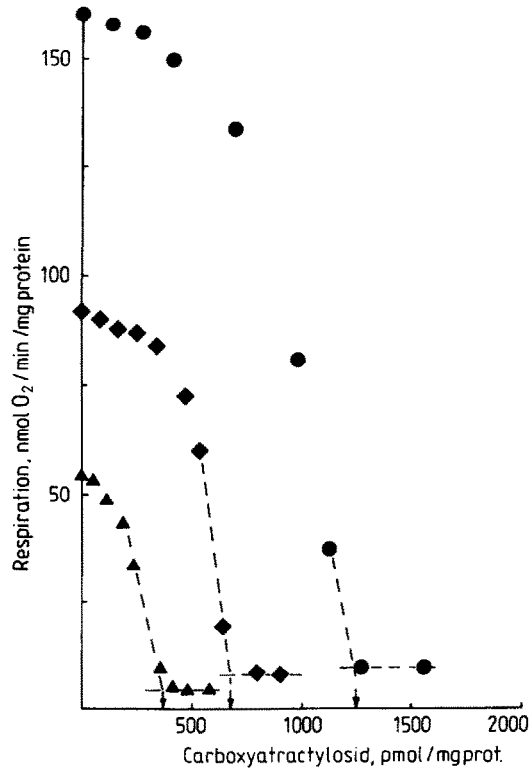

Fig. 2. Determination of adenine nucleotide translocase content by titration of active respiration with CAT. The mitochondria were incubated with the standard medium (without oligomycin) that was supplemented with 1 mM ATP and an excessive amount of hexokinase.

membrane compositions (for review see [17]). With protein-free phospholipid bilayers it had been demonstrated that LCFA are able to increase the membrane H^+ conductance though to a lesser extent than artificial protonophores [5,7,8]. Based on these studies, the conclusion was drawn that the translocation of deprotonated LCFA is the rate-limiting step in the process of H^+ conductance [5,8].

Table I

Adenine nucleotide translocase content and CAT sensitivity of oleate-stimulated respiration of mitochondria from rat liver, heart and kidney cortex tissue

Mitochondria	ANT content (pmol CAT/mg protein)	CAT	Slope (nmol O_2/min/ nmol oleate)	CAT sensitivity (in %)
RLM	298 ± 51	–	0.69	27.5
	(n = 8)	+	0.50	
RHM	1430 ± 170	–	1.33	67.7
	(n = 7)	+	0.43	
RKM	647 ± 50	–	1.25	54.4
	(n = 7)	+	0.59	

The ANT content was determined from different preparations, the number of which was put in parentheses. The slope was derived by linear regression from the types of experiments as shown in Fig. 1. The slope is presented as the mean of two experiments

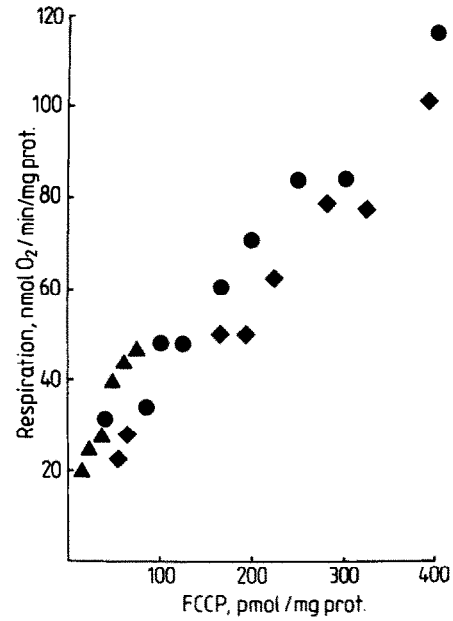

Fig. 3. Dose-response relationship between respiration and FCCP concentration with different types of mitochondria. RHM (0.8 mg/ml), RLM (1.4 mg/ml) and RKM (1.0 mg/ml) were incubated with the standard medium.

Acknowledgements: The author is grateful to Professor Dr W. Kunz and Dr E.N. Mokhova for stimulating discussions. Besides, the skillful technical assistance of Mrs H. Goldammer and the help of Mrs F. Gross in finishing the manuscript is gratefully acknowledged.

REFERENCES

[1] Wojtczak, L. (1976) J. Bioenerg. Biomembr. 8, 293–311.
[2] Rottenberg, H. and Hashimoto, K. (1986) Biochemistry 25, 1747–1755.
[3] Luvisetto, S., Pietrobon, D. and Azzone, G.F. (1987) Biochemistry 26, 7332–7338.
[4] Andreyev, A.Yu., Bondareva, T.O., Dedukhova, V.I., Mokhova, E.N., Skulachev, V.P. and Volkov, N.I. (1988) FEBS Lett. 226, 265–269.
[5] Andreyev, A.Yu., Bondareva, T.O., Dedukhova, V.I., Mokhova, E.N., Skulachev, V.P., Tsofina, L.M., Volkov, N.I. and Vygodina, T.V. (1989) Eur. J. Biochem. 182, 585–592.
[6] Schönfeld, P., Schild, L. and Kunz, W. (1989) Biochim. Biophys. Acta 977, 266–272.
[7] Gutknecht, J. (1987) Biochim. Biophys. Acta 898, 97–108.
[8] Gutknecht, J. (1988) J. Membr. Biol. 106, 83–93.
[9] Steinbrecht, I. and Kunz, W. (1970) Acta Biol. Med. Germ. 25, 731–742.
[10] Schaller, M., Letko, G. and Kunz, W. (1978) Acta Biol. Med. Germ. 37, 31–38.
[11] Hartung, K.-J., Jung, K., Minda, R. and Kunz, W. (1985) Biomed. Biochim. Acta 44, 1435–1443.
[12] Sokolowski, A. and Liese, W. (1973) Z. Med. Labortechn. 14, 247.
[13] Klingenberg, M. (1980) J. Membr. Biol. 56, 97–105.
[14] Weidemann, M.J., Erdelt, H. and Klingenberg, M. (1970) Eur. J. Biochem. 182, 585–592.
[15] Klingenberg, M., Giebe, K. and Scherer, B. (1975) Eur. J. Biochem. 52, 351–363.
[16] Skulachev, V.P. (1963) Proc. V. Int. Biochem. Congr. Moscow 5, 365–374.
[17] Daum, G. (1985) Biochim. Biophys. Acta 822, 1–42.