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Inhibitors of the ATP/ADP antiporter suppress stimulation of mitochondrial respiration and H^+ permeability by palmitate and anionic detergents

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The action of ATP/ADP-antiporter inhibitors upon the uncoupling effect of palmitate, detergents and 'classical' uncouplers has been studied. The uncoupling effect was estimated by stimulation of succinate oxidation and of H^+ permeability of rat liver mitochondria in the presence of oligomycin. It is shown that carboxyatractylate (CAtr) and pyridoxal 5-phosphate (PLP) suppress the uncoupling induced by palmitate and the anionic detergents SDS and cholate, but do not affect that induced by the cationic detergents CTAB, by the non-ionic detergent Triton X-100, as well as by the 'classical' uncouplers FCCP and DNP. The results are discussed in terms of a concept assuming that the ATP/ADP-antiporter facilitates the electrophoretic export of hydrophobic anions from mitochondria.

Fatty acid; Uncoupling; ATP/ADP-antiporter

1. INTRODUCTION

As was previously shown by our group [1–3], the stimulation of mitochondrial respiration and the membrane potential decrease by low concentrations of fatty acids are suppressed by CAtr and, to a lesser degree, by some other ATP/ADP-antiporter inhibitors and by its substrate, ADP. This effect, recently reproduced by Schönfeld [4,5], indicates that the ATP/ADP-antiporter is somehow involved in the fatty acid-induced uncoupling. We suggested [2,3] that it facilitates the export of anions of fatty acids from mitochondria. Such an event seems to be necessary for H^+ potential dissipation by fatty acids, since only the protonated (but not the anionic) form of fatty acid can easily traverse the membrane without assistance of any protein (for reviews, see [2,3]). Within the framework of this concept, the ATP/ADP-antiporter, very specific for hydrophilic anions (adenine nucleotides), loses its specificity with the increase in hydrophobicity. In agreement with such an assumption, we obtained an indication that the stimulation of mitochondrial respiration by low concentrations of the anionic detergent, SDS, is suppressed by CAtr [3]. It was also shown in other laboratories that hydrophobic anions (tetraphenyl borate, anionic detergents) inhibit the transport of adenine nucleotides by the antiporter [6,7].

In this paper, it is reported that the stimulation of controlled respiration and the H^+ permeability increase, induced by palmitate, SDS, and by another anionic detergent, cholate, are suppressed by CAtr and PLP, whereas the similar effects of cationic (CTAB) and non-ionic (Triton) detergents are resistant to these inhibitors of the ATP/ADP-antiporter.

2. MATERIALS AND METHODS

Isolation of rat liver mitochondria and polarographic measurements of oxygen consumption were performed as described previously [3]. The isolation mixture contained 0.25 M sucrose, 5 mM Mops and 2 mM EGTA, pH 7.4. A similar mixture was used as the incubation medium, but the EGTA concentration was reduced to 0.5 mM; temperature, 37°C.

H^+ permeability of the mitochondrial membrane was estimated by measuring the initial rate of pH change in the incubation medium upon the addition of HCl shifting pH by 0.12–0.15 [8]. In these experiments, the isolation medium contained 0.3 M sucrose, 0.5 mM Tris-HCl, 0.5 mM EGTA, pH 7.4 (mitochondria were suspended in medium without EGTA). The incubation medium contained 125 mM KCl, 100 μM EGTA, oligomycin (1 μg/ml), 1 mM $MgCl_2$, pH 7.4; temperature, 27°C. To obtain anaerobiosis, the incubation medium and mitochondrial suspension were bubbled with argon.

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Abbreviations: DNP, 2,4-*p*-dinitrophenol; BSA, bovine serum albumin; FCCP, *p*-trifluoromethoxycarbonylcyanide phenylhydrazone; Mops, morpholinopropane sulphonate; SDS, sodium dodecyl sulfate; CTAB, cetyltrimethylammonium bromide; PLP, pyridoxal 5-phosphate; CAtr, carboxyatractylate

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The following compounds were used in the experiments: fatty acid-free BSA, SDS, CAtr, succinate, Mops, oligomycin, cholic acid and palmitic acid (Sigma); ADP, rotenone, EGTA, CTAB, hexokinase, Tris (Serva); FCCP (Fluka); Triton X-100 (Merck); PLP (Reanal). Palmitic acid, rotenone and oligomycin were dissolved in twice-distilled ethanol. Sucrose was crystallized from a saturated solution in bidistilled water by adding twice-distilled ethanol; cholic acid was crystallized from a solution in 75% ethanol.

3. RESULTS

Table I shows the effect of the ATP/ADP-antiporter inhibitors on the palmitate- or detergent-induced stimulation of succinate oxidation in rat liver mitochondria under respiratory control conditions. In all the samples, oligomycin (1 $\mu g \cdot mg$ protein^{-1}) and 1×10^{-6} M rotenone were present. Concentrations of palimitate and detergents, causing approximately two-fold stimulation of the controlled respiration, were used. As inhibitors of the ATP/ADP-antiporter, 1×10^{-6} M CAtr and 2 mM PLP were tested. In the same experiments, it was found that 1.5×10^{-7} M CAtr and 0.5 mM PLP caused 50% inhibition of the state 3 respiration.

Table I

Effect of CAtr and PLP upon stimulation of respiration by palmitate and detergents in rat liver mitochondria under respiratory control conditions

Exp.	Additions	Respiration, n mol $O_2 \cdot min^{-1} \cdot mg$ protein^{-1}
(1)	-	16.5 ± 1.0
	Palm	29.0 ± 1.0
	Palm + CAtr	24.0 ± 1.5
	Palm + CAtr + DNP	72.0 ± 2.5
(2)	-	16.5 ± 0.1
	Palm	30.0 ± 0.5
	Palm + PLP	23.5 ± 0.5
	Palm + PLP + CAtr	21.0 ± 1.5
	Palm + PLP + CAtr + DNP	65.0 ± 2.0
(3)	-	16.5 ± 1.5
	SDS	31.0 ± 1.0
	SDS + CAtr	26.0 ± 0.2
	SDS + CAtr + DNP	71.5 ± 0.2
(4)	-	16.0 ± 0.5
	SDS	34.5 ± 2.0
	SDS + PLP	26.0 ± 2.0
	SDS + PLP + CAtr	21.0 ± 1.0
	SDS + PLP + CAtr + DNP	63.0 ± 1.5
(5)	-	15.0 ± 0.5
	Cholate	28.0 ± 0.5
	Cholate + CAtr	23.0 ± 0.5
	Cholate + CAtr + DNP	61.0 ± 3.5
(6)	-	14.0 ± 0.5
	CTAB	29.0 ± 1.0
	CTAB + PLP	27.0 ± 1.0
	CTAB + PLP + CAtr	27.0 ± 1.0
	CTAB + PLP + CAtr + DNP	70.0 ± 1.0
(7)	-	15.0 ± 0.1
	Triton	27.5 ± 1.0
	Triton + PLP	26.5 ± 1.5
	Triton + PLP + CAtr	29.0 ± 2.0
	Triton + PLP + CAtr + DNP	56.5 ± 1.0

Incubation mixture, 0.25 M sucrose, 5 mM Mops, pH 7.4, 0.5 mM EGTA, 5 mM succinate, 1×10^{-6} M rotenone, oligomycin (1 $\mu g \cdot mg$ protein^{-1}) and rat liver mitochondria (1 mg protein$\cdot ml^{-1}$). Additions, 1.5×10^{-5} M palmitate, 0.15 mM SDS, 1.2 mM cholate, 5×10^{-5} M CTAB, Triton X-100 (0.14 $mg \cdot ml^{-1}$), 1×10^{-6} M CAtr, 2 mM PLP and 2×10^{-5} M DNP. Values are means ± SE (n = 5-7).

As seen in Table I, CAtr and PLP appear to be inhibitory for respiration stimulated by palmitate and anionic detergents, i.e. SDS and cholate, being without effect on that stimulation by cationic (CTAB) and non-ionic (Triton X-100) detergents. In experiments with cholate, PLP was less effective than CAtr. In all the cases, the addition of 2×10^{-5} M DNP after CAtr and PLP strongly increased the respiration rate.

Table II summarizes the effects of CAtr on the increase in mitochondrial H^+ permeability caused by palmitate, SDS and FCCP. It shows that CAtr suppresses the permeability increase induced by palmitate and SDS, but not by FCCP. As to the H^+ permeability in the absence of uncouplers and detergents, it was not affected by CAtr. At the same time, this permeability could be lowered by anaerobiosis. The addition of palmitate or SDS increased the H^+ permeability of anaerobic mitochondria, though less than under aerobic conditions. This effect was abolished by CAtr. H^+ permeability in the presence of FCCP under anaerobiosis was almost as high as that under aerobiosis.

4. DISCUSSION

The above data indicate that the ATP/ADP-antiporter-mediated uncoupling activity is inherent not only in fatty acids, but also in synthetic anionic detergents, i.e. SDS and cholate. The uncoupling effect

Table II

Effect of CAtr upon the H^+ permeability increase induced by palmitate, SDS and FCCP in rat liver mitochondria

Additions	The initial rate of pH increase after acid pulse (pg ion $H^+ \cdot min^{-1} \cdot mg$ protein^{-1})	
	Aerobiosis	Anaerobiosis
-	165 ± 20	60 ± 10
CAtr	165 ± 10	-
Palm	350 ± 30	105 ± 15
Palm + CAtr	240 ± 20	40 ± 5
SDS	390 ± 40	135 ± 20
SDS + CAtr	210 ± 30	60 ± 10
FCCP	620 ± 50	540 ± 30
FCCP + CAtr	630 ± 40	-

For details, see section 2. Additions, mitochondria (1 mg protein$\cdot ml^{-1}$), 2×10^{-5} M palmitate, 0.2 mM SDS, 5×10^{-8} M FCCP and 1×10^{-6} M CAtr.

of these compounds was estimated by (i) stimulation of the controlled respiration and (ii) by an increase in the H^+ permeability of mitochondrial membrane. Both parameters were increased by a factor 2 in the presence of the investigated concentrations of palmitate or anionic detergents. This increase was shown to be lowered by the addition of the ATP/ADP-antiporter inhibitors, CAtr and PLP. It is essential that the inhibitor concentrations causing this effect did not arrest the stimulation of either respiration or H^+-permeability by the artificial protonophorous uncouplers DNDP and FCCP, as well as by the cationic detergent CTAB and the non-ionic detergent Triton X-100.

Concentration of palmitate, SDS and cholate inducing two-fold stimulation of respiration were found to be about 1.5×10^{-5} M, 1.5×10^{-4} M and 1.2×10^{-3} M, respectively. Thus, the efficacy of the fatty acid, which is regarded as a natural uncoupler [2], appears to be much higher than that of synthetic anionic detergents.

Two mechanisms of the ATP/ADP-antiporter-mediated uncoupling by fatty acids were considered [3]. (i) The allosteric modification of the antiporter by fatty acid binding results in the appearance of H^+ permeability via the antiporter. (ii) The antiporter facilitates the electrophoretic export of fatty acid anions from mitochondria.

In the former case, we ought to assume that the ATP/ADP-antiporter can translocate not only nucleotide anions but also H^+. In the latter case, the ATP/ADP-antiporter is still regarded as an anion carrier, which is postulated to be of high specificity for hydrophilic anions (ATP and ADP), but of low specificity for hydrophobic ones. According to this hypothesis, the antiporter allows fatty acid to operate as a protonophorous uncoupler moving into mitochondria in the protonated form (RCOOH) but returning to the extramitochondrial space in the deprotonated form ($RCOO^-$). Experiments on planar bilayers and proteoliposomes have clearly shown that protonated fatty acids can easily penetrate the phospholipid membrane, whereas their anions cannot [2,3]. So, translocation of $RCOO^-$ should be the rate-limiting step in the fatty acid-induced uncoupling.

Within the framework of this hypothesis, the ATP/ADP-antiporter is competent in the export of dodecyl sulfate and cholate anions, whereas their protonated forms are imported when moving via the phospholipid bilayer.

At first glance, it seems surprising that dodecyl sulfate can be protonated at neutral pH, since its pK_a is rather acidic. However, there are some reasons to suggest that this pK_a value can strongly increase in the membrane-water interface. As it was recently shown by Sankaram et al. [9], pK_a of stearic acid carboxyl in the phospholipid-water interface is as high as 8. The sorption of extrinsic proteins on the phospholipid bilayer surface is found to induce a further increase in pK_a (in some cases up to 9.6).

It is noteworthy that SDS and cholate differ from fatty acid in the anionic group and in the hydrophobic residue, respectively. The fact that both are competent in the ATP/ADP-antiporter-mediated uncoupling shows that the only requirement to the uncoupler of such a type is to be a hydrophobic anion.

The degree of CAtr and PLP inhibition of the respiration or H^+ permeability stimulated by palmitate or anionic detergents was about 50%. This value proved to be much higher when heart mitochondria, instead of liver ones, were used (not shown in tables). This fact is in agreement with the much higher content of the ATP/ADP-antiporter in heart, as first observed by Schönfeld [5].

The uncoupling effect of such 'classical' protonophores as FCCP (at any concentration) and DNP (at high concentrations) proved to be CAtr- and PLP-resistant. This could be expected, for the phospholipid membrane is rather permeable for both the anionic and the protonated forms of these uncouplers [2].

As to the uncoupling by the cationic detergent CTAB, and by the non-ionic detergent Triton X-100, it also seems to be ATP/ADP-antiporter-independent. Unable to bind protons, these agents can hardly by protonophores. Their uncoupling effect may be due to some non-specific damage of the mitochondrial membrane.

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