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Effect of the Cationic Detergent CTAB on the Involvement of ADP/ATP Antiporter and Aspartate/Glutamate Antiporter in Fatty Acid-Induced Uncoupling of Liver Mitochondria

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Abstract—The influence of the positively charged amphiphilic compound cetyltrimethyl ammonium bromide (CTAB) on palmitate- and laurate-induced uncoupling and on carboxyatractylate and glutamate recoupling effects in liver mitochondria have been studied. CTAB (40 μM) in the presence of 3 mM $MgCl_2$ had little (if any) effect on the palmitic acid-stimulated respiration of mitochondria; the glutamate recoupling effect increased, and the carboxyatractylate recoupling effect decreased to the same degree with the combined effect (about 80%) remaining unchanged. Thus, CTAB decreases the ADP/ATP antiporter involvement and increases to the same extent the aspartate/glutamate antiporter involvement in the fatty acid-induced uncoupling. The carboxyatractylate and glutamate recoupling effects were less pH dependent in the presence of CTAB than in its absence. These data could be interpreted with the assumption that fatty acid anions are more accessible to the ADP/ATP antiporter and their neutral forms are more accessible to the aspartate/glutamate antiporter, and that CTAB changes the relative anion carrier involvement in the fatty acid-induced uncoupling as it forms neutral complexes with fatty acid anions.

Key words: uncoupling, fatty acids, glutamate, carboxyatractylate, aspartate/glutamate antiporter, ADP/ATP antiporter, CTAB, liver mitochondria

The uncoupling mechanism of long-chain fatty acids in liver mitochondria involves the ADP/ATP antiporter [1-3], the aspartate/glutamate antiporter [2-8], and the dicarboxylate carrier [9, 10]. The contribution of the first two carriers to uncoupling is most significant (up to 80% of the overall uncoupling effect of fatty acids) [7, 8]. The involvement of the above-mentioned carriers in the uncoupling effect of fatty acids is now beyond question, but it remains unclear whether these proteins interact with each other in the course of the uncoupling process.

According to a hypothesis of Skulachev, the involvement of anionic carriers in the uncoupling process consists both in their assistance of fatty acid anion transfer and in their protonation in the active center of the carrier [2]. Once protonated, fatty acids are supposed to pass easily across a membrane along the concentration gradient (by the flip-flop mechanism) [2, 3, 10].

Some data suggest that the ADP/ATP antiporter and aspartate/glutamate antiporter act as parallel but independent pathways for mitochondrial $\Delta\mu H^+$ dissipation [8]. One of the arguments supporting this assumption is that the recoupling effects of the ADP/ATP antiporter inhibitor, carboxyatractylate, and aspartate/glutamate antiporter substrate, glutamate (or aspartate) are additive [5] and independent of the order of addition. These data were ascribed to the random allocation of fatty acid molecules between the carriers in the presence of magnesium ions and to the absence of redistribution in the course of uncoupling [8].

However, our data presented below do not support the assumption that the ADP/ATP and aspartate/glutamate antiporters act independently when they are involved in fatty acid-mediated uncoupling.

Abbreviations: DNP) 2,4-dinitrophenol; TPP^+) tetraphenylphosphonium; CTAB) cetyltrimethyl ammonium bromide; EGTA) ethylene glycol-bis(2-aminoethyl ether)-N,N,N′,N′-tetraacetic acid; $\Delta\mu H^+$) proton electrochemical potential drop across the inner mitochondrial membrane.

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1. In some experiments in low ion strength media containing no magnesium ions, glutamate exhibits recoupling activity only added after carboxyatractylate, whose recoupling effect increases significantly in the presence of glutamate [4]. A similar dependence was also observed in heart mitochondria [11].

2. Contributions of various anion transporters can change in opposite directions when incubation medium pH value is changed: aspartate/glutamate antiporter prevails at pH 7.0, whereas ADP/ATP antiporter prevails at pH 7.8. However, the combined recoupling effect of carboxyatractylate and glutamate therewith remains unchanged [7, 8].

3. Hydrophobic TPP^+ cations added to mitochondria [8, 12] induce the same opposite changes as occur when the incubation medium pH is lowered to 7.0. Opposite changes are caused by the negatively charged amphiphile lauryl sulfate [12]. However, the combined recoupling effect of carboxyatractylate and glutamate remains unchanged in this case too [7, 8]. These effects were ascribed to local pH changes at the lipid/water interface resulting from an appearance of either positive or negative extra charges on the membrane surface [12].

In the studies on the involvement of the ADP/ATP and aspartate/glutamate antiporters in the uncoupling effect of fatty acids, comparison of the magnesium ion, TPP^+, and lauryl sulfate effects mentioned above with that of a positively charged amphiphile, CTAB, appears to be reasonable. We found earlier that in low ion strength media containing no magnesium ions, CTAB enhanced the recoupling effect of glutamate but impaired that of carboxyatractylate [6]. Further experiments with CTAB were interrupted when unclear data for the CTAB effect on the uncoupling activity of lauric acid were realized.

The present work is devoted to the study of the influence of CTAB on the extents of ADP/ATP and aspartate/glutamate antiporter involvement in the uncoupling effects of lauric and palmitic acid. The results might be explained from the supposition that anionic fatty acid forms are readily accessible to the ADP/ATP antiporter, whereas their neutral forms are readily accessible to the aspartate/glutamate antiporter, and that CTAB alters the relative involvement of the anionic carriers in the uncoupling mechanism of fatty acids because it binds fatty acid anions to form neutral complexes.

MATERIALS AND METHODS

Mitochondria were isolated from the livers of white rats (180-220 g) as described previously in detail [13]. Mitochondrial suspension (70 mg mitochondrial protein per ml) was stored on ice. Mitochondrial protein was measured by the biuret method.

Mitochondrial respiration was monitored at 25°C using an LP-9 polarograph equipped with a Clark-type electrode. The following incubation medium was used: 250 mM sucrose, 10 mM potassium succinate, 2 μM rotenone, 0.5 mM EGTA, 3 mM $MgCl_2$, and 10 mM MOPS-KOH, pH 7.4. Oligomycin (2 μg/ml) was added immediately after the mitochondria (1 mg/ml).

Recoupling effect (%) was determined as the ratio of the respiration rate decrease after recoupling agent addition in the presence of fatty acid to the respiration rate change after fatty acid addition.

The following chemicals were used: MOPS, lauryl sulfate, palmitic acid, lauric acid, oligomycin, potassium succinate, potassium glutamate, carboxyatractylate, and fatty-acid-free BSA (Sigma, USA); rotenone and EGTA (Serva, Germany); CTAB and $MgCl_2$ (Merck, Germany).

Sucrose was recrystallized from its aqueous solution by precipitation with ethanol. Solutions of palmitic and lauric acids (20 mM) in ethanol and lauryl sulfate (40 mM) in the incubation medium were used. Ethanol was distilled twice.

RESULTS

Since fatty acids are amphiphiles, they are able, in addition to the uncoupling of oxidative phosphorylation, to increase the negative charge density on the mitochondrial membrane surface [14]. The positively charged amphiphile (cationic detergent) CTAB can decrease the negative surface potential, and magnesium ions have a similar effect [14]. In our previous studies, we demonstrated the effect of CTAB on the recoupling activities of carboxyatractylate and glutamate in the absence of magnesium ions [6]. If the effect of CTAB results from the decrease in negative surface potential only, it is expected to weaken markedly in the presence of magnesium ions.

When 3 mM $MgCl_2$ is present in the incubation medium without any other additions, CTAB stimulates mitochondrial respiration by about 25-30% (Table 1). Under these conditions, added palmitic acid increases the mitochondrial respiration rate by approximately equal amounts independently of the presence or absence of CTAB. Subsequent addition of carboxyatractylate leads to partial suppression of respiration, and CTAB decreases this effect. In contrast, the inhibitory effect of glutamate on respiration is more expressed in the presence of CTAB (Table 1A). Under the influence of CTAB the recoupling effect of carboxyatractylate decreases from 41 ± 2 to 17 ± 4% and the recoupling effect of glutamate increases from 39 ± 2 to 64 ± 3%. The combined recoupling effect of these substances is about 80% and remains unchanged when CTAB is added. We previously reported closely approximating changes in the recoupling effects of carboxyatractylate and glutamate caused by CTAB in the absence of $MgCl_2$ [6]. The data suggest that magnesium ions in the incubation medium have no effect on the abil-

Table 1. Effect of CTAB on respiration of liver mitochondria in the presence of palmitic (A) and lauric (B) acids and subsequent additions of carboxyatractylate and glutamate. The experimental conditions are given in "Materials and Methods". Additions: 40 μM CTAB, 30 μM palmitic (Palm) or lauric (Laur) acid, 0.5 μM carboxyatractylate (CAtr), 2 mM glutamate (Glu), and 50 μM DNP. Data are given in terms of mean value ± SEM (n = 5-6)

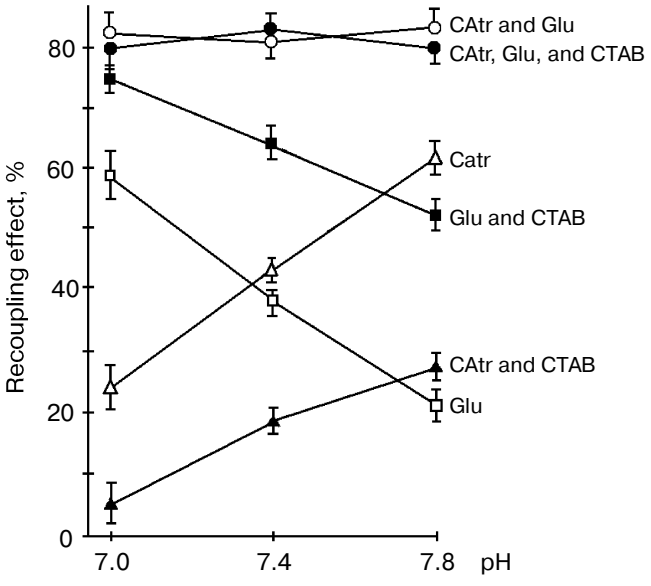
Additions		Respiration rate, nmol O_2/min per mg protein	
		without CTAB	40 μM CTAB
A	—	10.8 ± 0.2	14.2 ± 0.9
	Palm	30.4 ± 1.0	34.6 ± 1.5
	Palm and Catr	22.3 ± 0.9	31.2 ± 1.5
	Palm, CAtr, and Glu	14.8 ± 0.4	18.2 ± 0.5
	Palm, CAtr, Glu, and DNP	62.8 ± 1.6	65.8 ± 4.5
B	—	10.7 ± 0.3	13.3 ± 0.5
	Laur	26.1 ± 1.7	43.9 ± 2.2
	Laur and Catr	21.6 ± 1.4	41.4 ± 2.1
	Laur, CAtr, and Glu	13.8 ± 0.5	25.7 ± 1.9
	Laur, CAtr, Glu, and DNP	82.1 ± 4.2	77.7 ± 2.2

ity of CTAB to change the recoupling effects of carboxyatractylate and glutamate in opposite directions. On this basis, there is little likelihood that the effect of CTAB results only from the decrease in the negative surface potential on the mitochondrial membrane.

When palmitic acid is replaced by lauric acid, the recoupling effect of carboxyatractylate decreases and the recoupling effect of glutamate increases with no change in their combined recoupling effect [8]. It is instructive to consider how CTAB influences the recoupling effects of these substances under the uncoupling action of lauric acid. As Table 1B shows, lauric acid stimulates mitochondrial respiration to a greater extent in the presence of CTAB than in its absence, and in these circumstances carboxyatractylate does not inhibit respiration. Under the uncoupling action of lauric acid, CTAB has no effect on the recoupling activity of glutamate (51 ± 3 and 53 ± 6% in the absence and in the presence of CTAB, respectively), whereas the recoupling effect of carboxyatractylate decreases from 29 ± 2 to 7 ± 2%, and their combined recoupling effect also decreases from 80 ± 3 to 60 ± 4%.

The figure shows the data on the effect of CTAB on the incubation medium pH dependence of carboxyatractylate and glutamate recoupling effects under the uncoupling action of palmitic acid. As seen from the figure, CTAB, when added to mitochondria, increases the recoupling effect of glutamate and simultaneously decreases the recoupling effect of carboxyatractylate at all incubation medium pH values tested. Along with this, the recoupling effects of both carboxyatractylate and glutamate depend to a lesser extent on the incubation medium pH in the presence of CTAB. When the pH is increased from 7.0 to 7.8, the recoupling effect of carboxyatractylate increases by 38% in the absence of CTAB and by 23% in its presence. Under the same conditions, the recoupling effect of glutamate decreases by 37% in the absence of CTAB and by 22% in its presence. It should be noted also that the effect of CTAB on the recoupling effects of both carboxyatractylate and glutamate does not depend on the order they are added (data not shown).

The negatively charged amphiphile (anionic detergent) lauryl sulfate can uncouple oxidative phosphorylation in liver mitochondria by the same mechanism as fatty acids [8, 12]. However, its effective concentration is 160 μM, i.e., 5-fold higher than equally effective concentration of palmitic acid. As it is evident from our experiments, CTAB at 40 μM concentration has no effect on mito-



Effect of CTAB on the recoupling effects of glutamate, carboxyatractylate, and their combination in liver mitochondria under the uncoupling action of palmitic acid at various incubation medium pH values. The experimental conditions are described in "Materials and Methods". The additions are the same as in Table 1. The data are mean values ± SEM (n = 6)

Table 2. Effect of CTAB on the recoupling effects of carboxyatractylate and glutamate in liver mitochondria under the uncoupling action of lauryl sulfate at various pH values. The experimental conditions are described in "Materials and Methods". Additions: 160 μM lauryl sulfate; other additions are the same as in Table 1. Data are given as mean value ± SEM ($n = 6$)

Conditions	pH	Recoupling effect, %		
		CAtr	Glu	CAtr and Glu
Without CTAB	7.0	49 ± 3	31 ± 6	80 ± 4
	7.4	53 ± 2	30 ± 3	83 ± 4
40 μM CTAB	7.0	15 ± 3	61 ± 6	76 ± 7
	7.4	27 ± 4	49 ± 4	76 ± 5

chondrial respiration in the presence of 160 μM lauryl sulfate (data not shown). Along with this, CTAB 2-fold increases the recoupling effect of glutamate and 3.3-fold decreases the recoupling effect of carboxyatractylate at pH 7.0; the effect of CTAB on these recoupling activities decreases when pH increases to 7.4 (Table 2). Thus, under the uncoupling action of lauryl sulfate CTAB causes oppositely directed changes in the recoupling effects of carboxyatractylate and glutamate as effectively as it does under the uncoupling action of palmitic acid.

DISCUSSION

The experiments indicate that CTAB increases the stimulating effect of lauric acid on mitochondrial respiration and has little influence on the stimulating effect of palmitic acid. We suppose this difference results from higher mobility of the neutral CTAB–laurate complex compared to that of the CTAB–palmitate complex in the hydrophobic region of the membrane because of smaller size of the former, since the lauric acid molecule is shorter by four methylene groups. The mobile neutral CTAB–laurate complex is supposed to be able to cross the inner mitochondrial membrane, thus transporting lauric anion with no participation of anion carriers. A similar idea that the inner mitochondrial membrane permeability for fatty acids increases, because they bind hydrophobic cations giving neutral complexes, was previously reported by Schonfeld, who found that high TPP^+ concentrations increased the uncoupling effect of fatty acids [15].

When palmitic acid acts as uncoupler, CTAB decreases the recoupling effect of carboxyatractylate and increases the recoupling effect of glutamate. This effect of CTAB is equally significant both in the presence (the present study) and in the absence [6] of magnesium ions and, hence, is not caused by the decrease in negative surface potential on the mitochondrial membrane. Since the recoupling effects of carboxyatractylate and glutamate are indicative of the participation of ADP/ATP and aspartate/glutamate antiporters, respectively, in the uncoupling effect of fatty acids, we can state that CTAB causes oppositely directed and equal in magnitude changes in the extents of participation of these carriers in the uncoupling.

As mentioned in the introduction, some other amphiphilic compounds (TPP^+ and lauryl sulfate) also cause oppositely directed changes in the recoupling effects of carboxyatractylate and glutamate (aspartate) with no change in their combined recoupling effect [6, 8, 12]. We assumed previously that these effects are due to the lipid/water interface pH shift to the alkaline region under the action of TPP^+ (or to the acidic region under the action of lauryl sulfate), because these compounds can bind the charged groups of phospholipids and membrane proteins [12]. However, this supposition is unable to explain why these compounds have no effect on the palmitic acid-induced stimulation of respiration rate under our experimental conditions (Table 1 and figure) and why the combined recoupling effect of carboxyatractylate and glutamate is constant.

We propose the following alternative hypothesis that explains the effect of CTAB (as well as TPP^+ and low-concentrations of lauryl sulfate) on the recoupling activities of ADP/ATP-antiporter and glutamate/aspartate-antiporter substrates and inhibitors.

Mitochondrial fatty acid carriers are supposed to be involved not only in the fatty acid transfer from the matrix, but in the fatty acid protonation on the cytoplasmic side as well [2]; it is thought that both carboxyatractylate and glutamate suppress both of these processes. Aspartate/glutamate antiporter has been shown to carry out the electrogenic exchange of glutamate to

aspartate with simultaneous proton transfer from the intermembrane area to the matrix, thus the antiporter possesses a proton-transferring pathway [16].

ADP/ATP antiporter binds carboxyatractylate that carries four negative charges at neutral pH values [17], and aspartate/glutamate antiporter binds substrates (glutamate and aspartate), which carry two negative charges and one positive one. The polypeptide chain of the ADP/ATP antiporter has significantly more positively charged amino acid (arginine and lysine) residues than negatively charged ones, and four of them seem to be involved in carboxyatractylate binding [18, 19].

Aspartate/glutamate antiporter is less well understood; however, as can be suggested from the structures of its substrate, its active center contains at least two positive charges and one negative one. The difference between these carriers in charge structure is, in our mind, the main cause of fatty acid molecule redistribution between them, which occurs under the influence of TPP^+, lauryl sulfate, and CTAB and under the influence of changes in proton concentration.

We anticipate that the negatively charged group of aspartate/glutamate antiporter keeps the anionic groups of fatty acids away from the interaction with cationic groups of the active center (decreases the interaction rate), and therefore neutral fatty acids (or their neutral complexes) are more accessible to the glutamate/aspartate antiporter. In contrast, fatty acid anions are more accessible to the ADP/ATP antiporter. CTAB alters the ratio between charged and neutral fatty acid forms.

When CTAB at near-equimolecular ratio with fatty acid is added to mitochondria, an additional amount of neutral complexes appears and the free anion quantity decreases. As a result, there is an increase in fatty acid molecules that can interact with aspartate/glutamate antiporter with a simultaneous decrease in the quantity of fatty acid molecules that can interact with ADP/ATP antiporter. Because the overall amount of fatty acid molecules remains unchanged, CTAB has no effect on the overall uncoupling activity of fatty acids unless a new uncoupling pathways appears (as occurs for lauric acid), which are unrelated to the anion carriers discussed here.

The negatively charged amphiphile lauryl sulfate has an opposite to CTAB effect on the recoupling activities of carboxyatractylate and glutamate when it is applied at concentrations such that its uncoupling activity is not yet manifested [12]. We assume that the ability of this compound to decrease the extent of the involvement of the aspartate/glutamate antiporter in uncoupling and to increase that of ADP/ATP antiporter is due to the negatively charged complex formation between lauryl sulfate and neutral fatty acid molecule via the hydrogen bond between the oxygen atom of lauryl sulfate and the OH-group of the fatty acid. Complex formation between the neutral and anionic forms was discussed previously for various uncouplers [20]. It decreases the abundance of the fatty acid molecules accessible to aspartate/glutamate antiporter and increases those accessible to the ADP/ATP antiporter. Here too, the overall amount of fatty acid molecules is constant, so lauryl sulfate has no effect on the combined recoupling activity of carboxyatractylate and glutamate [12].

When higher (uncoupling) lauryl sulfate concentrations are applied, ADP/ATP antiporter begins to play the main role in the uncoupling process (Table 2 and [8, 12]). This is explainable because lauryl sulfate is a strong acid and is present only in its anionic form, which is, according to our hypothesis, predominantly accessible to the ADP/ATP antiporter. CTAB cations added form a complex with some of the lauryl sulfate anions, thus making them accessible to the aspartate/glutamate antiporter.

The data indicate that a similar mechanism exists for the effects of both protons and CTAB. Actually, decreasing the incubation medium pH to 7.0 results in the same oppositely directed changes in the extents of ADP/ATP antiporter and aspartate/glutamate antiporter involvement in the uncoupling process as when CTAB is added [7]; in both cases these changes may result from the decrease in the share of anionic fatty acid form. This mechanism is not realized when lauryl sulfate is used as the uncoupler because its anions are not protonated at neutral pH values. This provides an explanation for the fact that the pH dependence of the recoupling effects of both carboxyatractylate and glutamate is far less expressed under the uncoupling action of lauryl sulfate than under that of fatty acids [8, 12].

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