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Free Fatty Acids as Inducers and Regulators of Uncoupling of Oxidative Phosphorylation in Liver Mitochondria with Participation of ADP/ATP- and Aspartate/Glutamate-Antiporter

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Abstract—In liver mitochondria fatty acids act as protonophoric uncouplers mainly with participation of internal membrane protein carriers – ADP/ATP and aspartate/glutamate antiporters. In this study the values of recoupling effects of carboxyatractylate and glutamate (or aspartate) were used to assess the degree of participation of ADP/ATP and aspartate/glutamate antiporters in uncoupling activity of fatty acids. These values were determined from the ability of these recoupling agents to suppress the respiration stimulated by fatty acids and to raise the membrane potential reduced by fatty acids. Increase in palmitic and lauric acid concentration was shown to increase the degree of participation of ADP/ATP antiporter and to decrease the degree of participation of aspartate/glutamate antiporter in uncoupling to the same extent. These data suggest that fatty acids are not only inducers of uncoupling of oxidative phosphorylation, but that they also act the regulators of this process. The linear dependence of carboxyatractylate and glutamate recoupling effects ratio on palmitic and lauric acids concentration was established. Comparison of the effects of fatty acids (palmitic, myristic, lauric, capric, and caprylic having 16, 14, 12, 10, and 8 carbon atoms, respectively) has shown that, as the hydrophobicity of fatty acids decreases, the effectiveness decreases to a greater degree than the respective values of their specific uncoupling activity. The action of fatty acids as regulators of uncoupling is supposed to consist of activation of transport of their anions from the internal to the external monolayer of the internal membrane with participation of ADP/ATP antiporter and, at the same time, in inhibition of this process with the participation of aspartate/glutamate antiporter.

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

Long-chain free fatty acids have different effects on energy functions of mitochondria depending on their concentration and experimental conditions [1-4]. In the presence of calcium ions, fatty acids are able to induce nonspecific permeability of the mitochondrial inner membrane for hydrophilic compounds (pore opening), which is suppressed by chelating agents EGTA and EDTA, and in some cases, but not always, by cyclosporin A [3, 4]. Under pathological conditions involving accumulation of fatty acids, this effect on mitochondria is regarded as one of the causes of cell death by apoptosis and necrosis [4]. In the absence of calcium ions (in the presence of EGTA in the incubation medium), fatty acids in micromolar concentration induce uncoupling of oxidative phosphorylation by a protonophoric mechanism in animal mitochondria [1-4]. An important physiological function of this so-called "mild" uncoupling of mammals is heat production to maintain desired body temperature, as well as decrease in generation by mitochondrial of toxic reactive oxygen species [2, 4]. In liver mitochondria metabolite-transferring proteins of the inner mitochondrial membrane are involved in the protonophoric uncoupling effect of fatty acids: governing ADP to ATP (ADP/ATP antiporter) and glutamate to aspartate (aspartate/glutamate antiporter) exchange transport [2, 3, 5-8]. Their participation provides 70-80% of the uncoupling activity of palmitic and lauric acids [2, 3, 6-8]. Another portion of the uncoupling activity of these fatty acids (20-30%) is apparently catalyzed by a different mechanism, being due to a special system sensitive to cyclosporin A, but not related to cyclophilin D [9].

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It is assumed that participation of ADP/ATP and aspartate/glutamate antiporters in the uncoupling effect of fatty acids consists of the transfer of the fatty acid anion from the inner monolayer of the membrane to the outer one, where these anions are protonated and travel in the opposite direction without the help of proteins by the flip-flop mechanism, later releasing a proton into the matrix [2, 3].

The degree of participation of ADP/ATP and aspartate/glutamate antiporters in the uncoupling effect of fatty acids may vary considerably. It was that uncoupling mainly involves the aspartate/glutamate antiporter at pH 7.0, and it involves the ADP/ATP antiporter at pH 7.8 [7]. Addition of hydrophobic cetyltrimethylammonium cations to mitochondria leads to oppositely directed changes, similar to those after lowering the pH of the incubation medium from 7.8 to 7.0 [10]. However, addition of negatively charged amphiphilic lauryl sulfate to mitochondria increases the participation of ADP/ATP antiporter in uncoupling and, therefore, decreases the activity of aspartate/glutamate antiporter [11]. To explain the phenomena described above it was suggested that fatty acids in the anionic form are better available for ADP/ATP antiporter, and in neutral form are better suitable for aspartate/glutamate antiporter [10]. However, other data do not agree with this hypothesis. Thus, the degree of involvement of these anion transporters in the uncoupling was also established to depend on fatty acid chain length: after application of relatively short-chain capric or lauric acid, uncoupling involved aspartate/glutamate antiporter to a greater extent than the ADP/ATP antiporter, while the reverse situation was observed after application of long-chain palmitic acid [8].

Saturated fatty acids with different numbers of carbon atoms in the molecule are very different in solubility in lipids [12, 13]. For example, increasing length of the acyl chain by two carbon atoms leads to 14-fold increase in lipid/water partition coefficient of fatty acids [12, 13]. This suggests that when applying the same concentration, the content of long-chain fatty acids in the hydrophobic region of the mitochondrial inner membrane will be greater than the content of short-chain fatty acids. It is interesting to speculate that the degrees of participation of ADP/ATP and aspartate/glutamate antiporters in uncoupling depend on the number of fatty acid anions in the hydrophobic inner membrane and not on the ratio of anionic and neutral forms, as thought previously [10]. This allows us to consider the fatty acids not only as uncouplers of oxidative phosphorylation, but also as regulators of this process.

The purpose of this study was to clarify the role of fatty acids in liver mitochondria as regulators of induced uncoupling with the participation of ADP/ATP and aspartate/glutamate antiporters. To achieve this goal it was necessary to clarify how the ability of glutamate and carboxyatractylate to inhibit uncoupling action of palmitic or lauric acids depends on the concentration of these uncouplers. The data obtained may be considered as evidence that increasing concentration of palmitic and lauric acid raises the participation of ADP/ATP antiporter in the uncoupling and reduces the participation of aspartate/glutamate antiporter in the uncoupling by the same amount. This suggests that fatty acids act not only as inducers of uncoupling with the participation of the carriers, but also as regulators of this process.

MATERIALS AND METHODS

Mitochondria from the liver of mature white male rats weighing 220-250 g were isolated by conventional differential centrifugation with subsequent separation of endogenous fatty acids with fatty acid-free BSA as described in detail previously [6]. The isolation medium contained 250 mM sucrose, 1 mM EGTA, 5 mM Mops-KOH, pH 7.4. The mitochondrial protein concentration was determined by the biuret method with BSA used as standard. During the experiments, the suspension of mitochondria (60-70 mg of mitochondrial protein in 1 ml) was stored on ice in a narrow tube (Eppendorf, Germany). Mitochondrial respiration was recorded using a Clark-type oxygen electrode and LP-9 polarograph in a thermostatted cell at 25°C. The incubation medium contained 250 mM sucrose, 5 mM succinic acid, 3 mM $MgCl_2$, 0.5 mM EGTA, 10 mM Mops-KOH, pH 7.4. To determine uncoupling action of fatty acids immediately after the addition of the mitochondria (~1.0 mg/ml), we added rotenone (2 μM) and oligomycin (2 μg/ml) into the polarographic cell, followed 2 min later by the studied fatty acid at the concentration indicated on the figures, followed by 1 μM carboxyatractylate 1.5 min later, then 2 mM glutamate 1.5 min later, and 50 μM 2,4-dinitrophenol another 1.5 min later. Special experiments showed that these concentrations of carboxyatractylate and glutamate inhibited the uncoupling effect of fatty acids to the maximum extent, and 2,4-dinitrophenol caused maximal stimulation of the mitochondrial respiration.

Membrane potential ($\Delta\psi$) was estimated by the distribution of TPP^+, the concentration of which was recorded with a TPP^+-sensitive electrode [14] at 25°C. In these experiments we further added 1.6 μM TPP^+ to the incubation medium and later added 20 nM nigericin after addition of the mitochondria. Nigericin was necessary in order to convert ΔpH in $\Delta\psi$. Special experiments showed that nigericin concentration of 20 nM was optimal, since its further increase does not increase $\Delta\psi$ either in the absence or in the presence of palmitic acid. The value of $\Delta\psi$ was calculated taking into account non-energy-dependent binding of TPP^+ with mitochondria according to the Nernst equation [14]. The volume of matrix was assessed as 1 μl/mg protein, which is average volume of liver mitochondria inaccessible to sucrose [15]. The abil-

ity of carboxyatractylate and glutamate to increase $\Delta\psi$ (recoupling effect) was expressed as percentage, and it was defined as the ratio of $\Delta\psi$ increase caused by one of these recoupling agents to reduction of $\Delta\psi$ magnitude caused by palmitic acid.

To quantify the uncoupling activity of fatty acids, as has been demonstrated previously [16], we used the magnitude of respiratory stimulation by this fatty acid (J_U), defined as the difference between the rate of mitochondrial respiration (nmol O_2/min per mg protein) before and after the addition of fatty acid. The J_U value was considered consisting of three parts – one sensitive to carboxyatractylate (J_A), one sensitive to glutamate (J_G), and one insensitive to both of these reagents [16]. As has been justified earlier, the first two components give quantitative description of, respectively, the participation of ADP/ATP antiporter and aspartate/glutamate antiporter in the uncoupling, and the third one corresponds to the participation in the uncoupling of some structure insensitive to the action of carboxyatractylate and glutamate [16]. The value of J_A was determined as the difference between the rate of mitochondrial respiration (nmol O_2/min per mg protein) in the presence of fatty acid before and after addition of carboxyatractylate, and the value of J_G as the difference between the rate of mitochondrial respiration (nmol O_2/min per mg protein) in the presence of fatty acid and carboxyatractylate before and after the addition of glutamate. The component of the uncoupling activity that is sensitive either to carboxyatractylate or glutamate (J_{AG}) was defined as the sum of J_A and J_G. Specific uncoupling activity of fatty acids (V_U) and the component of this activity, sensitive to carboxyatractylate and glutamate (V_{AG}), were defined as the quotient after division of J_U and J_{AG} values, respectively, by the concentration of fatty acid.

We used Mops, fatty acids (palmitic, myristic, lauric, capric, and caprylic), oligomycin, succinic acid, potassium glutamate, potassium aspartate, carboxyatractylate, diethylpyrocarbonate, fatty acid-free BSA from Sigma (USA), rotenone and EGTA from Serva (Germany), tetraphenylphosphonium chloride, 2,4-dinitrophenol, sucrose, and KCl from Fluka (Germany), and $MgCl_2$ and KOH from Merck (Germany). We used solutions of fatty acids in double-distilled ethanol.

RESULTS

We used saturated fatty acids in the experiments, which differ from unsaturated ones by higher resistance to peroxidation [17]. Most of the research was conducted with palmitic acid, which is one of the most common natural fatty acids [1]. It was shown previously [16] that the dependence of mitochondrial respiration rate on the concentration of palmitic acid is close to linear in the range of concentrations from 0 to 40 μM. As seen from Fig. 1, palmitate at concentration of 15 μM increases mitochondrial respiration 1.83-fold, and at concentration of 35 μM it increases 3-fold. The subsequent addition of carboxyatractylate and glutamate leads to partial inhibition of respiration, which indicates their ability to inhibit the uncoupling action of fatty acids or, in other words, their recoupling action [6, 7]. Under palmitic acid concentration of 15 μM glutamate inhibits respiration to a greater degree than carboxyatractylate, while under palmitic acid concentration of 35 μM carboxyatractylate is more effective than glutamate (Fig. 1). When you change the sequence of additions of these recoupling agents, first glutamate and then carboxyatractylate, one can observe a similar pattern in changes of their effects with increasing concentration of fatty acids (data not shown).

It is well proven that the suppression of fatty acids uncoupling action by carboxyatractylate indicates the involvement of the ADP/ATP antiporter in uncoupling, and suppression by glutamate (aspartate or diethylpyrocarbonate) involves the aspartate/glutamate antiporter [2, 3, 5-8]. To quantify the degree of participation of ADP/ATP and aspartate/glutamate antiporters in the

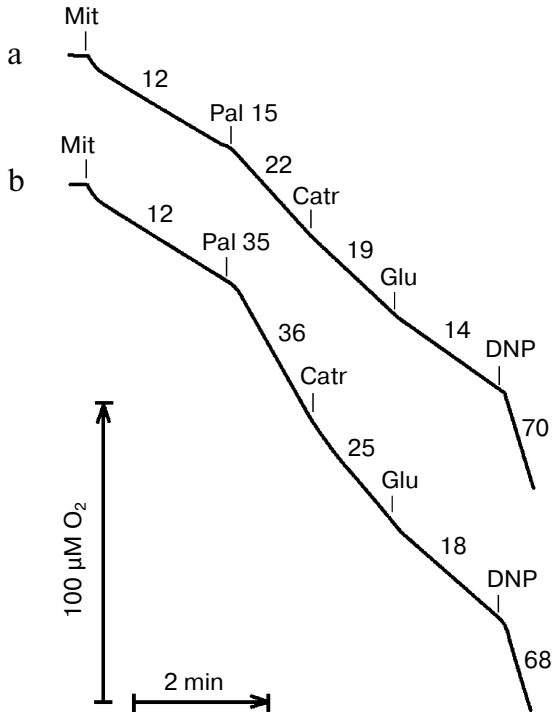

Fig. 1. Comparison of effect of carboxyatractylate and glutamate on stimulation of liver mitochondria respiration by palmitic acid in concentrations of 15 (a) and 35 μM (b). The experimental conditions are listed in "Materials and Methods". Mit, mitochondria (1 mg of protein); Pal 15 and Pal 35, palmitic acid (15 and 35 μM); Catr, carboxyatractylate (1 μM); Glu, glutamate (2 mM); DNP, 2,4-dinitrophenol, 50 μM. Here and in Fig. 2 data from a typical experiment are shown that were obtained from one preparation of mitochondria. Similar results were obtained in three more independent experiments.

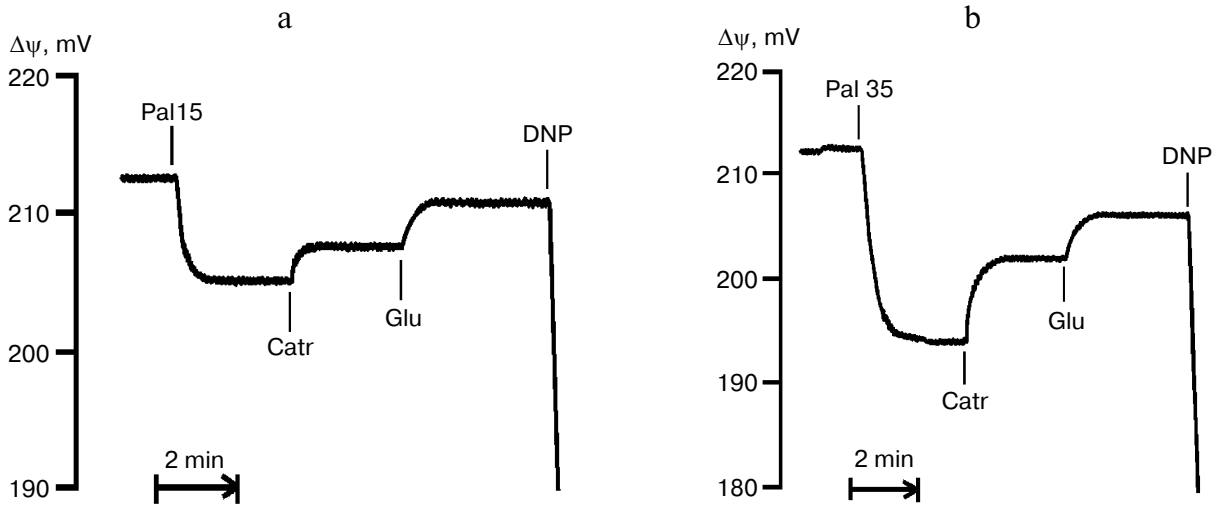

Fig. 2. Comparison of the effect of carboxyatractylate and glutamate on Δψ of liver mitochondria in the presence of palmitic acid in concentrations of 15 (a) and 35 μM (b). The experimental conditions are listed in "Materials and Methods". DNP, 100 μM 2,4-dinitrophenol; other additives are similar to those indicated in the legend to Fig. 1.

uncoupling effect of fatty acids, we used the values of recoupling effects of carboxyatractylate and glutamate (or aspartate), respectively [7, 8]. These values can be determined from the degree of suppression of mitochondrial respiration that was stimulated by fatty acids [7, 8]. In this study, it was found that as concentration of palmitate increases from 15 to 35 μM, recoupling effect of carboxyatractylate increases from 33 ± 1 to 43 ± 1% (n = 4), while recoupling effect of glutamate decreases from 44 ± 1 to 27 ± 1% (n = 4). Under these conditions the ratio of recoupling effects of carboxyatractylate and glutamate increased from 0.76 ± 0.02 to 1.58 ± 0.04 relative units (n = 4). Similar results were obtained when glutamate was replaced with another substrate of the aspartate/glutamate antiporter, aspartate, as well as after the addition of a nonspecific inhibitor of this transporter, diethylpyrocarbonate (data not shown).

Figure 2 represents data obtained from experiments to determine the effect of carboxyatractylate and glutamate on the transmembrane electric potential (Δψ), reduced by palmitic acid in concentration of 15 μM (Fig. 2a) and 35 μM (Fig. 2b). As can be seen, the addition of palmitic acid to mitochondria leads to a greater decrease in Δψ at concentration of 35 μM. Addition of carboxyatractylate and glutamate leads to an increase in Δψ (recoupling effect), which is consistent with earlier data [6]. Under palmitic acid concentration of 15 μM glutamate acts more efficiently than carboxyatractylate, while increasing the concentration of the uncoupler to 35 μM makes carboxyatractylate more efficient than glutamate. Changing the addition sequence of these recoupling agents to first glutamate and then carboxyatractylate, we observed a similar pattern in changes of their effects with increasing concentration of fatty acids (data not shown). Recoupling effects of carboxyatractylate and glutamate can be determined by the degree of Δψ increase that was previously reduced by palmitic acid. It was established that increasing concentration of palmitate from 15 to 35 μM makes the recoupling effect of carboxyatractylate increase from 31 ± 3 to 42 ± 3% (n = 4), while the recoupling effect of glutamate decreases from 41 ± 4 to 26 ± 2% (n = 4). Under these conditions the ratio of recoupling effects of carboxyatractylate and glutamate increases from 0.76 ± 0.13 to 1.62 ± 0.06 (n = 4).

Our studies have shown that an increase in the concentration of palmitic acid from 15 to 35 μM increases the degree of participation of ADP/ATP antiporter in uncoupling and accordingly decreases the participation of aspartate/glutamate antiporter in uncoupling. These data were obtained with two independent estimation methods of fatty acid uncoupling action. In all subsequent experiments, uncoupling action of fatty acids was estimated by the degree of stimulation of mitochondrial respiration.

The dependence of the uncoupling activity of palmitic acid on its concentration in the range from 0 to 35 μM is close to linear (Fig. 3, line *1*). Lauric acid differs from palmitic acid in its lesser hydrophobicity [12, 13]. The dependence of the uncoupling activity of lauric acid on its concentration in the range from 0 to 60 μM is also close to linear (Fig. 3, line *2*). According to the definition of uncoupling activity (section "Materials and Methods") in the absence of added fatty acids this value is 0. The data presented in Fig. 3 also shows that the tangent of the line when uncoupling with palmitic acid is 30% greater than the same value when uncoupling with lauric acid. Due to the fact that in this case the slope of the line can be regarded as specific uncoupling activity of fatty acid (V_U) [16], these data suggest that the uncoupling activity of

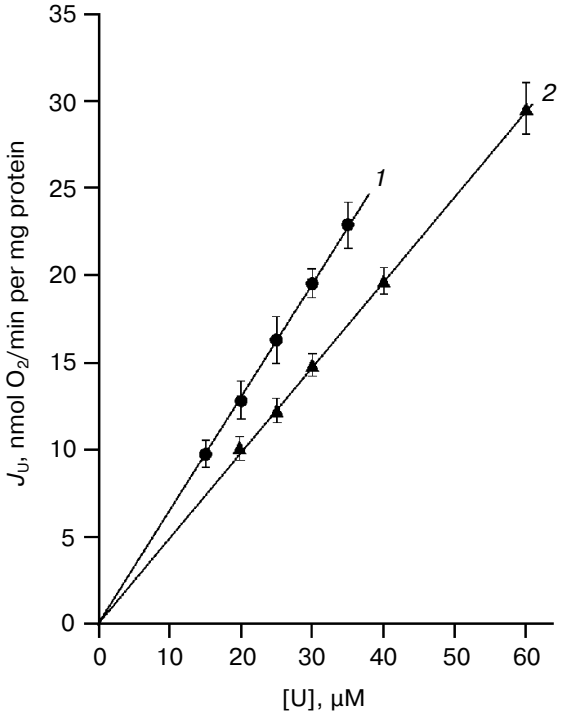

Fig. 3. Dependence of the uncoupling activity (J_U) of palmitic (*1*) and lauric (*2*) acids in liver mitochondria on the concentrations ([U]) of these uncouplers. Mean values ± standard errors ($n = 4$) are shown.

palmitate is higher than the uncoupling activity of laurate by 30%. This slight excess is consistent with published data obtained by comparing the uncoupling activity of various fatty acids [18, 19].

There is linear dependence of the component of the uncoupling activity that is sensitive to carboxyatractylate and glutamate (J_{AG}) on the concentration of palmitic and lauric acids (Fig. 4). In this case, the tangent of a line may be regarded as sensitive to carboxyatractylate and glutamate component of the specific uncoupling activity of fatty acid (V_{AG}). The intersection of the experimental lines with the ordinate axis is not at the zero point. This can be explained by the fact that endogenous fatty acids also participate in uncoupling, and their action is also inhibited by carboxyatractylate and glutamate. Uncoupling activity of endogenous fatty acids that is sensitive to glutamate and carboxyatractylate, determined at this point, has a value of 1.0 ± 0.2 nmol O_2/min per mg protein ($n = 4$). Evidently, the endogenous fatty acids remained in the mitochondria in small amounts even after the purification procedure with BSA. Apparently, there is a considerable amount of long-chain fatty acids among them (e.g. stearic acid), as their transition from phospholipid bilayer into the aqueous phase for binding with BSA happens much slower than for short-chain acids [20]. It should be noted that stearic acid has a significantly lower uncoupling activity when compared with palmitic and lauric acids [18, 19].

The concentration of endogenous fatty acids can be expressed as the concentration of palmitic or lauric acid ($[U_0]$). In the first case, this value is 2.30 ± 0.31 μM ($n = 4$), while in the second case it is 2.97 ± 0.43 μM ($n = 4$). This difference is due to the fact that palmitic acid is more effective than lauric acid as uncoupler and, consequently, lower concentrations are necessary to achieve the uncoupling activity equal to the activity of endogenous fatty acids.

We have already noted that we used recoupling effects of carboxyatractylate and glutamate (or aspartate), respectively, to quantify the participation of ADP/ATP and aspartate/glutamate antiporters in the uncoupling effect of fatty acids. The recoupling effect of carboxyatractylate can be defined as the ratio of J_A and J_U, and the recoupling effect of glutamate can be defined as the ratio of J_G and J_U. It should be noted that the values of J_A and J_G were determined while taking into consideration the contribution of endogenous fatty acids to the uncoupling, while the value of J_U was calculated not considering this fact. Therefore, to correctly determine the recoupling effects of carboxyatractylate and glutamate at all concentrations of fatty acids it is necessary to add the value of uncoupling activity of endogenous fatty acids (J_{0U}) to the values of J_U. This value is determined by the formula $J_{0U} = V_U [U_0]$, and it amounts to 1.5 ± 0.1 and 1.5 ±

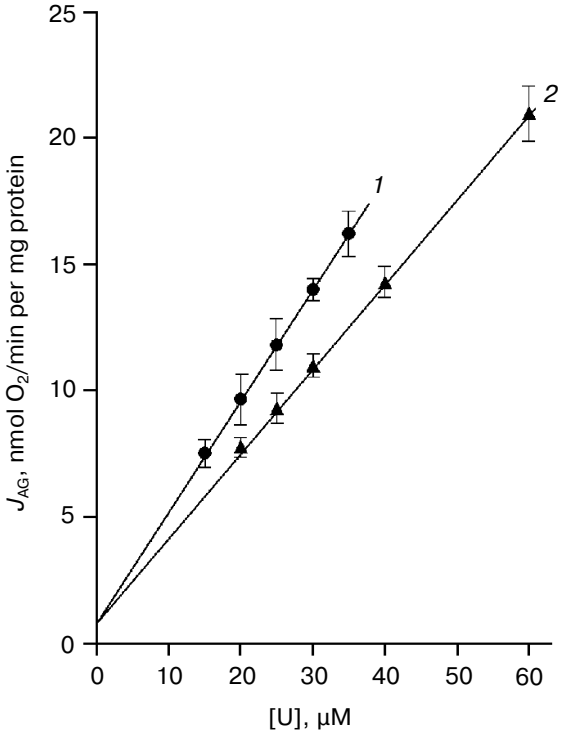

Fig. 4. Dependence of the uncoupling activity component sensitive to carboxyatractylate and glutamate (J_{AG}) of palmitic (*1*) and lauric (*2*) acids in liver mitochondria on the concentrations ([U]) of these uncouplers. Mean values ± standard errors ($n = 4$) are shown.

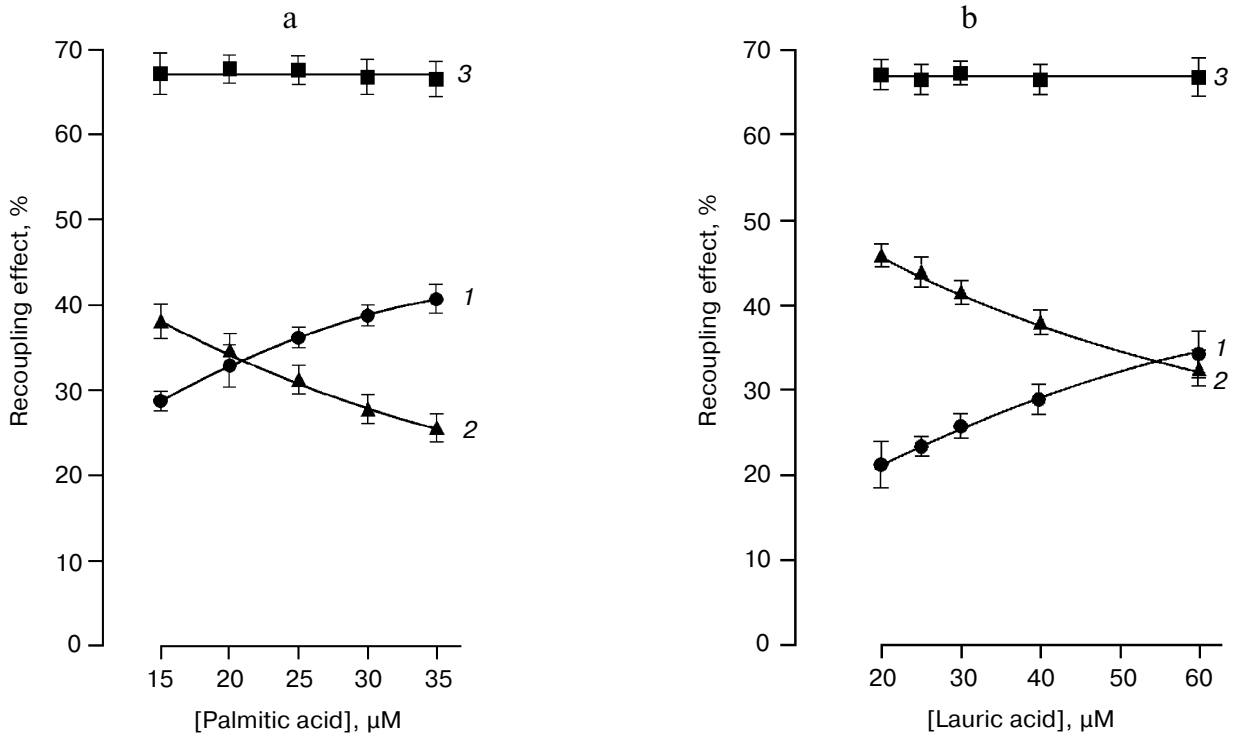

Fig. 5. Comparison of the recoupling effects of carboxyatractylate (*1*), glutamate (*2*) and their combined recoupling effect (*3*) under the uncoupling action of palmitic (a) and lauric (b) acids at different concentrations in liver mitochondria. Mean values ± standard errors ($n = 4$) are shown.

0.2 nmol O_2/min per mg protein ($n = 4$) when uncoupling with palmitic and lauric acids, respectively.

As seen from Fig. 5a, the recoupling effect of carboxyatractylate rises and the recoupling effect of glutamate lowers by the same amount without changing the total recoupling effect of these agents with increasing concentrations of palmitic acid. Similar results were obtained when replacing palmitic acid with lauric acid (Fig. 5b). Therefore, the level of participation of ADP/ATP antiporter in uncoupling increases and the level of participation of aspartate/glutamate antiporter in uncoupling decreases to the same extent with increasing concentration of palmitic and lauric acid. At the same time, the degree of participation of insensitive to carboxyatractylate or glutamate structure (or structures) in uncoupling does not depend on the concentration of fatty acids (Fig. 5). These data can be viewed as evidence that fatty acids are not only inducers of uncoupling of oxidative phosphorylation, but also regulators of this process with participation of ADP/ATP and aspartate/glutamate antiporters.

The dependence of the ratio of recoupling effects of carboxyatractylate and glutamate (equivalent to ratio of J_A and J_G) on the concentrations of palmitic and lauric acids was studied to compare the efficiency of these fatty acids as regulators of uncoupling. As seen from Fig. 6, there is a linear dependence of (J_A/J_G) on the concentrations of palmitic and lauric acids. It is noteworthy that the intersection of the experimental line with the ordinate axis is observed at the same point when uncoupling with either of the two acids (Fig. 6). This point is determined by the ratio of recoupling effects of carboxyatractylate and glutamate in the absence of added fatty acid. Since this value is not 0, we can assume that there are some endogenous fatty acids in the inner membrane of mitochondria involved in the regulation of uncoupling together with the added fatty acids.

The data presented in Fig. 6 can be described by the empirical equation:

$$\frac{J_A}{J_G} = K_e[U_0] + K_e[U]. \quad (1)$$

Here [U] is the concentration of fatty acids; $[U_0]$ is the concentration of endogenous fatty acids that act as regulators of uncoupling, expressed as the concentration of palmitic or lauric acids; K_e is coefficient of proportionality. We call this coefficient, which shows the effectiveness of a fatty acid as a regulator of uncoupling, the efficiency factor.

Based on the experimental data and Eq. (1), the efficiency factor can be determined using two different concentrations of fatty acids by the equation:

$$K_e = \frac{R_2 - R_1}{[U]_2 - [U]_1}. \quad (2)$$

Comparison of the efficiency factor (K_e), the specific uncoupling activity (V_U) and its component, sensitive to carboxyatractylate and glutamate (V_{AG}) after treatment with saturated fatty acids: palmitic (C16), myristic (C14), lauric (C12), capric (C10), and caprylic (C8) in liver mitochondria

Fatty acids	K_e		V_U		V_{AG}	
	Abs	%	Abs	%	Abs	%
C16	40 ± 3	100 ± 7	648 ± 29	100 ± 4	483 ± 40	100 ± 8
C14	34 ± 3	85 ± 7	755 ± 49	117 ± 8	565 ± 34	117 ± 7
C12	15 ± 2	37 ± 5	501 ± 14	77 ± 2	365 ± 14	76 ± 3
C10	2.3 ± 0.3	5.7 ± 0.7	105 ± 7	16 ± 1	76.2 ± 4.9	16 ± 1
C8	0.28 ± 0.02	0.70 ± 0.05	18.0 ± 0.7	2.8 ± 0.1	12.9 ± 0.6	2.7 ± 0.1

Note: The experimental conditions are listed in "Materials and Methods". Absolute values (Abs) of K_e – relative units/mM, of V_U and V_{AG} – nmol O_2/min per 1 μM of fatty acid. Mean values ± standard errors (n = 4) are shown.

Here the symbols R_2 and R_1 were introduced to denote the value of (J_A/J_G) at the concentrations $[U]_2$ and $[U]_1$ of the fatty acid, respectively.

Some ensuing experiments were conducted to compare the effects of saturated fatty acids: palmitic, myristic, lauric, capric and caprylic with 16, 14, 12, 10, and 8 carbon atoms in the molecule, respectively. Efficiency factor values were calculated according to Eq. (2). We also determined values of specific uncoupling activity (V_U) for these fatty acids and its component sensitive to carboxyatractylate and glutamate (V_{AG}). We established that in the series of saturated fatty acids the decrease in their hydrophobicity corresponds to decrease in all studied variables (see table). The extent of decrease can be compared by expressing it in relative units. As can be seen from the table, with decrease in the hydrophobicity of fatty acids the efficiency factor decreased to a greater extent than the value of uncoupling activity.

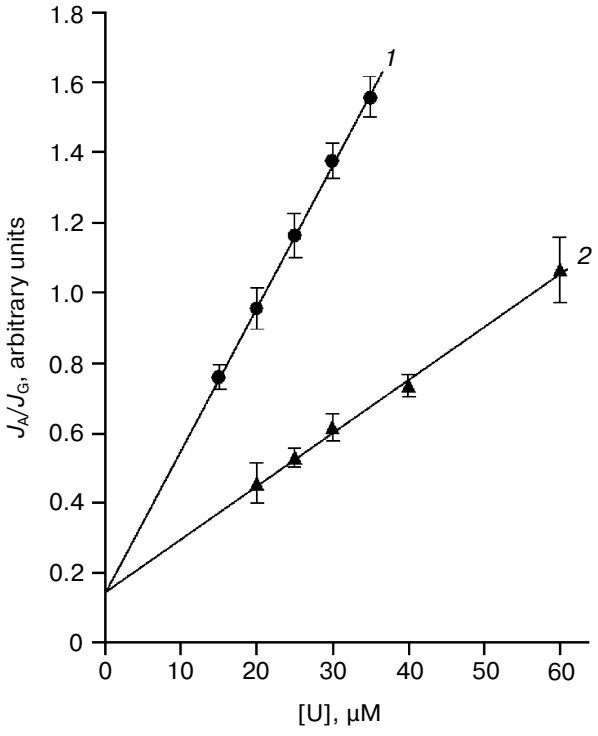

Fig. 6. Dependence of the recoupling effects ratio of carboxyatractylate and glutamate (J_A/J_G) under uncoupling with palmitic (*1*) and lauric (*2*) acids in liver mitochondria on concentrations ([U]) of these uncouplers. Mean values ± standard errors (n = 4) are shown.

DISCUSSION

Thus, results of the studies suggest that fatty acids can be regarded not only as uncouplers of oxidative phosphorylation, but also as regulators of this process. When the concentration of a fatty acid increases, the degree of participation of ADP/ATP antiporter in uncoupling rises and the degree of participation of aspartate/glutamate antiporter in uncoupling lowers by the same amount (Fig. 5). Previously, similar changes in the degree of participation of these anion transporters in uncoupling were observed when increasing pH of the incubation medium [7, 10]. This phenomenon was explained by the fact that increasing pH increases the ratio of anionic and neutral forms of fatty acids, while the anionic forms of fatty acids are more available to the ADP/ATP antiporter, and the neutral forms are better suited for the aspartate/glutamate antiporter [10]. But the results obtained in this work cannot be explained by this hypothesis. It is unlikely that increased concentration of a fatty acid increases the proportion of its anions in the presence of excess magnesium ions. Indeed, according to published data [21], increasing

the number of fatty acids carboxyl groups on the membrane surface leads to a local increase in pH in the lipid/water interphase, which in turn increases the share of neutral, not anionic, forms of the fatty acids.

As mentioned in the introduction, the addition of surplus anionic groups of lauryl sulfate to the mitochondria leads to an increase in the degree of participation of ADP/ATP antiporter in uncoupling and, correspondingly, to a decrease in the role of aspartate/glutamate antiporter [11]. Apparently, similar changes in the degree of participation of these transporters in the uncoupling with increasing concentrations of fatty acids observed in this study are due to the action of the negative charges of their carboxyl groups, while the neutral molecules of fatty acids are not effective. One of the arguments in favor of this assumption is that the nonionic detergent Triton X-100 (a neutral amphiphilic compound) does not influence the recoupling effects of carboxyatractylate and glutamate when uncoupling with palmitic acid, in contrast with the anionic detergent lauryl sulfate and the cationic detergent cetyltrimethylammonium bromide (unpublished data).

Fatty acids, being amphiphilic compounds, are capable of increasing the density of negative charges on the surface membranes of mitochondria [1]. We can assume that the regulatory effect of fatty acids is caused by the action of their negatively charged carboxyl groups on the ADP/ATP and aspartate/glutamate antiporters. As mentioned in the introduction, the role of these transporters in uncoupling is to transfer the fatty acid anions from the inner monolayer of the membrane to the outer one [2, 3]. Apparently, the anions of fatty acids activate this process with the participation of ADP/ATP antiporter and inhibit it with participation of aspartate/glutamate antiporter. Some analogy can be drawn with classical enzymological phenomena of substrate activation in relation to the ADP/ATP antiporter and substrate inhibition with respect to aspartate/glutamate antiporter.

This work shows that the effectiveness of fatty acids as regulators of uncoupling decreases with a decrease in their hydrophobicity. It can be assumed that anions of various fatty acids, being in the hydrophobic region of membrane, are equally effective as regulators of uncoupling. Therefore, the above difference in regulatory effects can only be explained by better solubility in lipids of more hydrophobic fatty acids when compared with less hydrophobic ones. Under the same conditions the efficiency of fatty acids as uncouplers of oxidative phosphorylation is reduced to a lesser extent. It is known that the uncoupling effect of fatty acids is associated with their transition from one monolayer of the inner membrane to the other [2, 3]. Short-chain fatty acids, when compared with long-chain ones, have higher mobility in the hydrophobic membrane [20]. Consequently, when the same number of molecules is present in the hydrophobic membrane, uncoupling activity of short-chain fatty acids may be higher than that of long-chain fatty acids.

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