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Effect of Ethanol on the Palmitate-Induced Uncoupling of Oxidative Phosphorylation in Liver Mitochondria

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Abstract—The effect of ethanol on the uncoupling activity of palmitate and recoupling activities of carboxyatractylate and glutamate was studied in liver mitochondria at various Mg^{2+} concentrations and medium pH values (7.0, 7.4, and 7.8). Ethanol taken at concentration of 0.25 M had no effect on the uncoupling activity of palmitic acid in the presence of 2 mM $MgCl_2$ and decreased the recoupling effects of carboxyatractylate and glutamate added to mitochondria either just before or after the fatty acid. However, ethanol did not modify the overall recoupling effect of carboxyatractylate and glutamate taken in combination. The effect of ethanol decreased as medium pH was decreased to 7.0. Elevated concentration of Mg^{2+} (up to 8 mM) inhibits the uncoupling effect of palmitate. Ethanol eliminates substantially the recoupling effect of Mg^{2+} under these conditions, but does not influence the recoupling effects of carboxyatractylate and glutamate. It is inferred that ADP/ATP and aspartate/glutamate antiporters are involved in uncoupling function as single uncoupling complex with the common fatty acid pool. Fatty acid molecules gain the ability to migrate under the action of ethanol: from ADP/ATP antiporter to aspartate/glutamate antiporter on addition of carboxyatractylate and in opposite direction on addition of glutamate. Possible mechanisms of fatty acid translocation from one transporter to another are discussed.

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

Free long-chain fatty acids play an important role in oxidative metabolism not only as substrates for oxidation, but also as natural uncouplers of oxidative phosphorylation participating in non-retractile thermogenesis and regulation of energy coupling under various physiological and pathological conditions in homoiothermic animals [1, 2]. A peculiar uncoupling (protonophore) effect of fatty acids in liver is mediated mainly by transporter proteins located in the inner mitochondrial membrane: ADP/ATP and aspartate/glutamate antiporters [2-7]. According to our studies, the total contribution of these proteins in uncoupling is 80% [6, 7]. Another constitutive part of the uncoupling is probably the participation of dicarboxylate transporter [4, 8] in this process as well as fatty acids, which are weak uncoupling protonophores [9-11].

The uncoupling activity of fatty acids can be controlled in different ways. The first way is to change the number of transporter proteins involved in the uncoupling, as it has been demonstrated in the case of ADP/ATP antiporter expression under hyperthyroidism, when the increased number of these transporters in mitochondria as a response to triiodothyronine injection is accompanied by an increase in the uncoupling of the constitutive part inhibited by carboxyatractylate, a ligand for this transporter [12]. The second way is to modulate the uncoupling effect of fatty acids by energy metabolism intermediates, such as glutamate, aspartate, cysteine sulfonate, and malate [5, 6, 13, 14].

Other ways for control over the uncoupling effect of fatty acid are possible too. One of them is the changing the number of fatty acid molecules interacting with ADP/ATP antiporter and aspartate/glutamate antiporter under various actions to mitochondria. In our previous studies we found that these transporters can act to the uncoupling in different directions, but their effect alters equally as medium pH changes: the contribution of ADP/ATP antiporter into the uncoupling increases as pH increases from 7.0 to 7.8, whereas the contribution of aspartate/glutamate antiporter decreases to the same extent [6, 7]. An addition of lipophilic tetraphenylphosphonium cations or amphiphilic cetyltrimethylammonium (CTAB) cations to mitochondria results in an increase in aspartate/glutamate antiporter contribution to the uncoupling and, to the same extent, in the decrease in ADP/ATP antiporter contribution [7, 15-

Abbreviations: DNP) 2,4-dinitrophenol; CTAB) cetyltrimethylammonium bromide.

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17]. Changes in opposite directions can be induced on addition of lauryl sulfate, a negatively charged amphiphilic compound [16]. Recently we proposed [17] that common mechanisms exist for H^+ and CTAB action on the uncoupling effect of fatty acids: in both cases there are equal but oppositely directed changes in the contribution of anion transporters to the uncoupling. On our opinion, these changes result from an increase in the level of fatty acids accessible for aspartate/glutamate antiporter and corresponding equal decrease in the level of fatty acids accessible for ADP/ATP-antiporter.

According to Academician Skulachev's hypothesis that is a favorite at present, anion transporters facilitate a transmembrane electrophoretic transfer of fatty acids, whereas the protonated forms of these acids freely cross the membrane along the gradient of their concentration (flip-flop) [2-4]. Fatty acid anions are supposed to translocate from transporter molecules on the outer membrane side to the site in phospholipid bilayer, where they are transformed into protonated form with subsequent flip-flop of the neutral molecules onto the opposite side of the membrane [4]. With this hypothesis in mind, one may suppose that fatty acids could be transferred by lateral diffusion from one transporter to another. This transfer of fatty acids seems to be possible on the interaction of transporter with its ligand: from ADP/ATP antiporter to aspartate/glutamate antiporter on the addition of carboxyatractylate and in opposite direction on the addition of glutamate. We found previously that the recoupling effect of carboxyatractylate in liver mitochondria develops only after the addition of glutamate against the uncoupling effect of stearate, whereas the recoupling effect of glutamate develops after its addition after carboxyatractylate [7]. One may get the impression of stearate molecules possessing a capability to move from one transporter to another without changing in their total uncoupling activity. However, the phenomenon found under uncoupling by stearate was not observed under uncoupling by other saturated fatty acids [7]. This difference is possibly because stearate taken in our experiments at concentration of 80 μM modifies mitochondrial membranes, thus expressing its detergent properties. Further experiments are needed using other fatty acids with uncoupling effect at lower concentrations in the presence of some other modifiers of biological membranes.

Ethanol attracted our attention because it is an effective and most studied modifier of biological membranes and can effect both phospholipid bilayer properties through elevation of its fluidity [18, 19] and modify properties of integral membrane proteins [20, 21]. When interacting with biological membranes, ethanol molecules are accumulated on the lipid/water interface surface, particularly between hydrophilic fragments of phospholipid molecules displacing water [22-24]. In our experiments ethanol usually serves as a solvent for fatty acids. We had to add so much ethanol to mitochondria as to achieve its 0.14 M final, relatively high, concentration in the reaction cell, when working with stearate due to its poor solubility [7]. Ethanol taken at this concentration can significantly elevate the fluidity of phospholipid bilayer membranes [19].

The presented work is devoted to study the effect of ethanol on uncoupling action of palmitate and change in ADP/ATP and aspartate/glutamate antiporter contribution to this process at various pH values and Mg^{2+} concentrations.

MATERIALS AND METHODS

Mitochondria were isolated from liver of white rats with body weight of 180-220 g, according to a previously described method [25]. The medium for isolation contained 250 mM sucrose, 2 mM EGTA, and 5 mM Mops-Tris, pH 7.4. Mitochondrial suspension (70 mg protein per ml) was stored on ice. Protein concentration was determined by the biuret method using BSA as a standard.

Mitochondrial respiration was detected at 25°C using a Clark oxygen electrode and LP-9 polarograph or, in some experiments, using an open platinum electrode. The incubation medium contained 250 mM sucrose, 5 mM potassium succinate, 10 mM KCl, 0.5 mM EGTA, 2 or 8 mM $MgCl_2$, and 5 mM Mops-Tris, pH 7.0, 7.4, or 7.8. Oligomycin (2 μg/ml) and rotenone (2 μM) were added immediately after the addition of mitochondria (1 mg/ml). The incubation medium for the detection of respiration rate of mitochondria in state 3 contained additionally 3 mM KH_2PO_4 and 2 mM $MgCl_2$ without oligomycin.

The uncoupling effect of palmitate was expressed in some experiments in arbitrary units as the extent of stimulation of mitochondrial respiration according to the formula $(J_U - J_0)/J_0$, where J_0 and J_U are the rates of mitochondrial respiration before and after palmitate addition, respectively.

Recoupling effects (%) of carboxyatractylate and glutamate were determined as ratios between the value of decrease in respiration rate on the addition of recoupling agent in the presence of fatty acid and the value of change in respiration rate on the addition of fatty acid.

Mops, Tris, palmitate, oligomycin, potassium succinate, potassium glutamate, carboxyatractylate, and fatty acid-free BSA were from Sigma (USA); rotenone and EGTA were from Serva (Germany); KCl and $MgCl_2$ were from Merck (Germany).

Sucrose was recrystallized by precipitation from ethanol solution. Palmitate stock solutions in ethanol were used (10 and 20 mM). Ethanol was distilled twice.

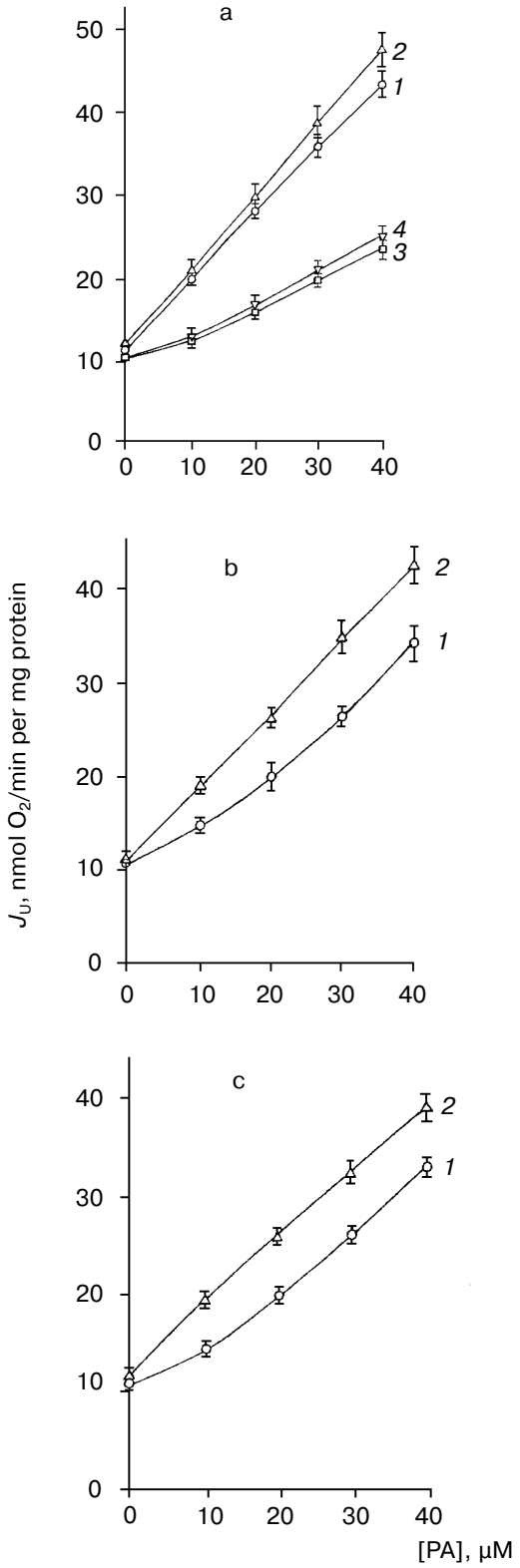

Fig. 1. Effect of ethanol on palmitate (PA)-induced stimulation of mitochondrial respiration (J_U): a) in the presence of 2 mM $MgCl_2$ as a single addition (*1* and *2*) or in the presence of 1 μM carboxyatractylate and 2 mM glutamate in combination (*3* and *4*); b) in the presence of 1 μM carboxyatractylate; c) in the presence of 2 mM glutamate. Means ± standard deviations are given ($n = 6$).

RESULTS

The influence of ethanol on the uncoupling effect of fatty acids was studied in the presence of 2 or 8 mM Mg^{2+}. We found previously that Mg^{2+} at concentration of 2 mM does not influence the uncoupling effect of palmitate (see also [15]). Ethanol concentration in the medium for incubation of mitochondria was 0.25 M in a series of experiments. The results of our experiments indicate that ethanol taken in this concentration does not influence the respiration of mitochondria in states 3 and 4, as well as in the presence of an uncoupler, 2,4-dinitrophenol (DNP), at optimum concentration of 50 μM. Ethanol begins to express an uncoupling effect at concentrations above 0.3 M (data not shown).

Figure 1 shows the effect of ethanol on the mitochondrial respiration at different palmitate concentrations in the absence and in the presence of carboxyatractylate and glutamate, added singly or together. Ethanol, carboxyatractylate, and glutamate were added to mitochondria in the presence of 2 mM $MgCl_2$ before the addition of palmitate (ethanol was present at the background concentration of 0.033 M owing to its addition into incubation medium with mitochondria together with oligomycin and rotenone). Carboxyatractylate and glutamate added separately against the background of 0.033 M ethanol inhibited mitochondrial respiration in the presence of 20 μM palmitate by 28 and 29%, respectively. As illustrated in Fig. 1a, 0.25 M ethanol does not influence mitochondrial respiration in the absence of palmitate and insignificantly (no more than 11%) stimulates the respiration in the presence of fatty acid (curves *1* and *2*). A preliminary addition of carboxyatractylate in combination with glutamate to mitochondria results in significant inhibition of uncoupling effect of palmitate (compare curves *1* and *3*), and addition of ethanol to mitochondria does not enhance the respiration in this case (curves *3* and *4*). A remarkable stimulation of mitochondrial respiration by ethanol in the presence of palmitate is only observed when carboxyatractylate (Fig. 1b) or glutamate (Fig. 1c) was added separately.

As illustrated in Fig. 2, in the absence of carboxyatractylate and glutamate (curve *1*) or in the presence of both (curve *4*), ethanol at concentration no more than 0.3 M has no influence on the uncoupling effect of palmitate. Ethanol at 0.2 M concentration remarkably enhances the uncoupling effect of palmitate against the background of glutamate alone (curve *2*) or carboxyatractylate alone (curve *3*), whereas the elimination of the recoupling effects of carboxyatractylate and glutamate was observed at 0.3 M concentration of ethanol.

The data shown in the Figs. 1 and 2 were obtained at pH 7.4. Ethanol substantially enhances the uncoupling effect of palmitate at pH 7.8 in the presence of carboxyatractylate or glutamate as well (data not shown). However, the effect of ethanol is weaker at pH 7.0 (data not shown).

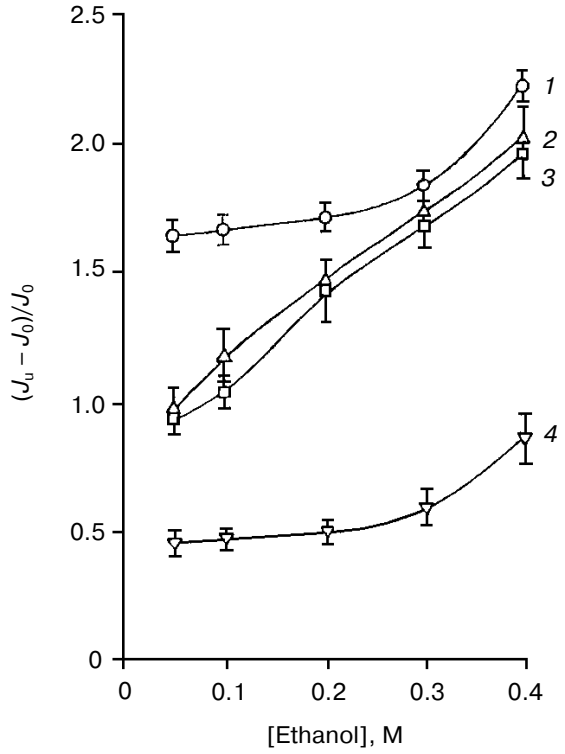

Fig. 2. Effect of ethanol on 20 μM palmitate (PA) uncoupling action ($(J_U - J_0)/J_0$) in the presence of 2 mM $MgCl_2$ in absence (*1*) or in presence of 2 mM glutamate (*2*), 1 μM carboxyatractylate (*3*), or 1 μM carboxyatractylate and 2 mM glutamate (*4*). Means ± standard deviations are given ($n = 5$).

The fatty acid stimulated capability of carboxyatractylate and glutamate to inhibit mitochondrial respiration can be considered as a recoupling effect. The data for the influence of ethanol on the recoupling effects of carboxyatractylate and glutamate are shown in Table 1. The order of addition of recoupling agents to mitochondria in the presence of 20 μM palmitate varied as follows: either carboxyatractylate first and then glutamate were added (*1*), or first glutamate then carboxyatractylate were added (*2*). As illustrated in Table 1, with 2 mM $MgCl_2$ in the absence of ethanol recoupling effects do not depend on the order of the additions at various pH values in the incubation medium. Ethanol diminishes substantially the recoupling effects of both carboxyatractylate and glutamate on addition of these substances after the fatty acid addition. However, in the case of another order of their addition, namely carboxyatractylate after glutamate or glutamate after carboxyatractylate, recoupling effects of these substances were substantially enhanced under the influence of ethanol. In both cases the total value of recoupling effects of carboxyatractylate and glutamate is not changed on ethanol addition.

A dependence of the ethanol effect described above on the medium pH attracted our attention: at pH 7.8, ethanol diminishes recoupling effects of carboxyatractylate by 77% and glutamate by 80%; these values comprise 60 and 58%, respectively, at pH 7.4, and only 37 and 32% at pH 7.0. The effect of ethanol can be supposed to depend on the ratio between deprotonated and protonat-

Table 1. Effect of ethanol on the recoupling activities of carboxyatractylate (CAtr) and glutamate (Glu) in liver mitochondria incubated with palmitate and 2 mM $MgCl_2$ at various pH values

pH	Experimental conditions	Sequence of additions	Recoupling effect, %		
			CAtr	Glu	CAtr + Glu
7.0	without ethanol	1	27 ± 2	53 ± 3	80 ± 3
		2	26 ± 2	50 ± 2	76 ± 3
	0.25 M ethanol	1	17 ± 2	61 ± 2	78 ± 4
		2	44 ± 2	34 ± 5	78 ± 4
7.4	without ethanol	1	42 ± 4	33 ± 5	75 ± 6
		2	44 ± 3	36 ± 4	80 ± 5
	0.25 M ethanol	1	17 ± 5	59 ± 7	76 ± 7
		2	60 ± 4	15 ± 5	75 ± 7
7.8	without ethanol	1	56 ± 3	22 ± 2	78 ± 3
		2	58 ± 2	20 ± 1	78 ± 2
	0.25 M ethanol	1	13 ± 3	64 ± 2	77 ± 4
		2	72 ± 2	4 ± 2	77 ± 3

Note: 20 μM palmitate, 1 μM carboxyatractylate, and 2 mM glutamate were added here and in Table 2; sequence of additions: *1*) addition of palmitate was followed by CAtr and then Glu additions; *2*) addition of palmitate was followed by Glu and then by CAtr. Means ± standard deviations are given ($n = 5$).

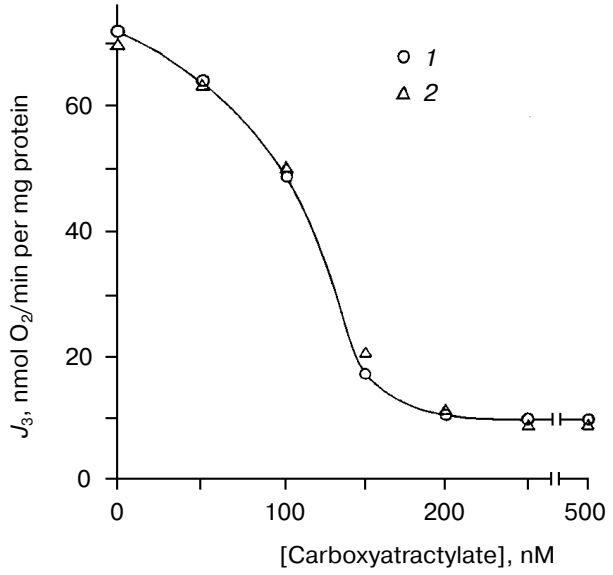

Fig. 3. Ethanol has no influence on mitochondrial respiration in state 3 (J_3) at various carboxyatractylate concentrations (without ethanol (*1*) and in the presence of 0.25 M ethanol (*2*)).

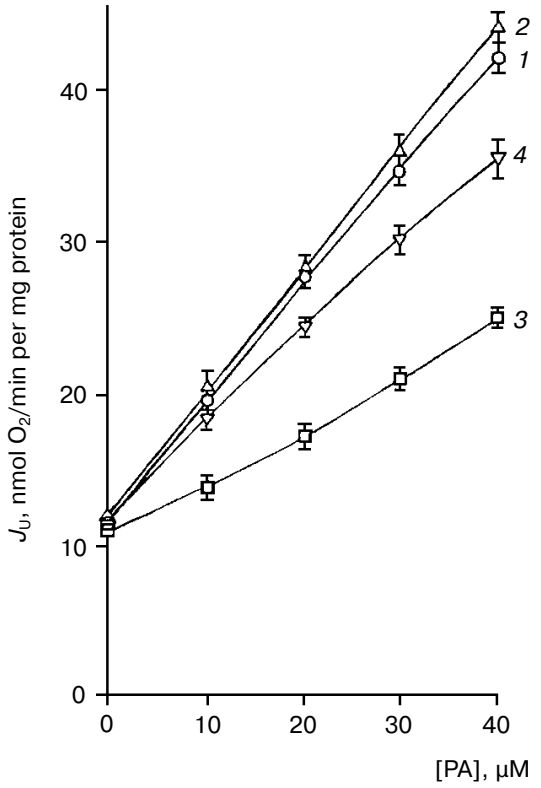

Fig. 4. Ethanol effect on palmitate-induced stimulation of mitochondrial respiration (J_U) (without ethanol (*1*, *3*) and with 0.25 M ethanol added (*2*, *4*)), in the presence of $MgCl_2$ at 2 mM (*1*, *2*) or 8 mM (*3*, *4*). Means ± standard deviations are given ($n = 5$).

ed forms of palmitate carboxyl group and/or some other acidic/basic groups on the surface of the mitochondrial membrane. The ratio between anionic and neutral forms of fatty acid carboxyl group can be calculated using the Henderson–Hasselbach equation for distinct pK_a on phospholipid membrane surface [26, 27]. Hence, the proportion of anionic form in the fatty acid pool can be determined. Our calculations demonstrated that the proportion of anionic form of carboxyl group with its pK_a taken as 7.3 (the mean pK_a 7.2 and 7.4 determined on the phospholipid membrane surface by different methods [26, 27]), yields 76% at pH 7.8, 56% at pH 7.4, and 33% at pH 7.0. These values are virtually equal to those given above characterizing the decrease in the carboxyatractylate and glutamate recoupling effects under the action of ethanol. Hence, the effect of ethanol changes proportionally to change in fatty acid anionic form proportion on the mitochondrial membrane surface.

Thus, the results obtained indicate that ethanol abates the recoupling effects of carboxyatractylate and glutamate on uncoupling by palmitate in the presence of 2 mM $MgCl_2$ provided that these compounds were added to mitochondria separately. One would suppose that this ethanol effect is due to its immediate influence on anionic transporters. In this case one would expect particularly that change in ADP/ATP antiporter properties led first to change in mitochondrial respiration rate in state 3 and second to change in inhibitory effect of carboxyatractylate depending on its concentration. As illustrated in Fig. 3, 0.25 M ethanol does not influence mitochondrial respiration independently of the presence of carboxyatractylate. Hence, the effect of ethanol is not due to its influence either on ADP/ATP antiporter or the capability of carboxyatractylate to interact with this transporter.

A decrease in mitochondrial respiration rate in the presence of palmitate occurs as $MgCl_2$ concentration is increased to 8 mM (Fig. 4), supporting known data on the ability of Mg^{2+} to diminish the decoupling effect of fatty acids [11]. An addition of 0.25 M ethanol diminishes the inhibitory effect of Mg^{2+}. Figure 5 illustrates the ability of ethanol to diminish the recoupling effect of Mg^{2+}. In the presence of 8 mM $MgCl_2$, ethanol enhances the decoupling effect of palmitate even at 0.2 M concentration, whereas with 2 mM $MgCl_2$ only 0.4 M is effective. A similar pattern was observed with carboxyatractylate and glutamate in combination: palmitate does not display uncoupling effect with 8 mM $MgCl_2$ without ethanol (Fig. 5).

In the presence of 8 mM $MgCl_2$, ethanol at concentration 0.25 M had no effect on the recoupling activities of carboxyatractylate and glutamate independently of the order of their addition to mitochondria. However, ethanol decreases the overall recoupling effect of carboxyatractylate and glutamate taken in combination, which reached almost 100% in the absence of ethanol (Table 2).

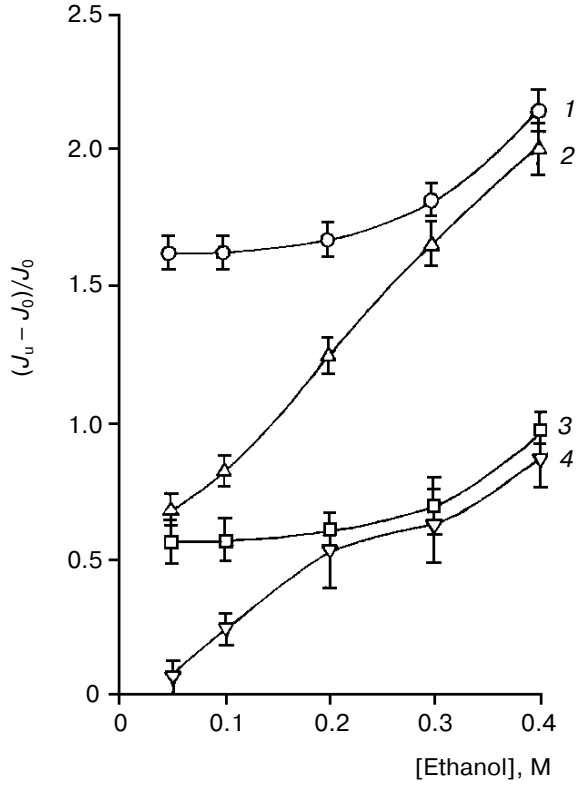

Fig. 5. Effect of ethanol on 20 μM palmitate (PA) uncoupling action ($(J_U - J_0)/J_0$) in the presence of 2 mM (*1*, *3*) or 8 mM $MgCl_2$ (*2*, *4*) and in absence (*1*, *2*) or presence (*3*, *4*) of 1 μM carboxyatractylate and 2 mM glutamate. Means ± standard deviations are given ($n = 5$).

DISCUSSION

Many amphiphilic substances, such as phospholipids and free fatty acids, are able to move along a membrane layer (lateral diffusion) [28]. Membrane proteins interfere with this lateral diffusion [28]. As we demonstrated earlier, in the presence of Mg^{2+} and all uncouplers tested, except stearate, the recoupling effects of carboxyatractylate and glutamate did not depend on the order of their addition [7]. We considered this data as evidence that fatty acids (except stearic acid) cannot migrate along the membrane layer between ADP/ATP and aspartate/glutamate antiporters [7]. It was found in the present work that in the presence of 2 mM $MgCl_2$ under the influence of ethanol and on uncoupling by palmitate diminishing of carboxyatractylate and glutamate recoupling effects occurs when these substances were added to mitochondria separately (Fig. 1 and Table 1). We anticipate that palmitate molecules gain the capacity to move under the influence of ethanol: from ADP/ATP antiporter to aspartate/glutamate antiporter on carboxyatractylate addition, and in the opposite direction on glutamate addition. The effect of ethanol may be due to either its capacity to elevate phospholipid membrane fluidity or direct influence anion transporters. The increase in phospholipid membrane fluidity is known to occur also at elevated temperatures [28], but, as demonstrated earlier, elevation of the temperature in the mitochondrial incubation medium from 25 to 37°C was not accompanied by decrease in carboxyatractylate recoupling effect [29]. Hence, the elevation of mitochondrial membrane fluidity should not be considered as a single cause for the ethanol effect observed. It is unlike that the effect of ethanol is due to its direct influence on anion transporters, since no alterations either in mitochondrial respiration rate in state 3 or in inhibiting effect of carboxyatractylate were observed under the influence of ethanol (Fig. 3).

The distribution of fatty acid molecules between ADP/ATP and aspartate/glutamate antiporters probably depends on the ratio of positive and negative charges in these transporters. Fatty acid anions would move to cationic groups of aspartate/glutamate antiporter on the interaction between tetravalent anions of carboxyatractylate and cationic groups of ADP/ATP antiporter, whereas fatty acid anions would move to cationic groups of ADP/ATP antiporter on neutralization of cationic groups of aspartate/glutamate antiporter by glutamate. In the

Table 2. Ethanol has no effect on recoupling effects of carboxyatractylate (CAtr) and glutamate (Glu) in liver mitochondria incubated with palmitate and 8 mM $MgCl_2$ at pH 7.4

Experimental conditions	Sequence of additions	Recoupling effect, %		
		CAtr	Glu	CAtr + Glu
Without ethanol	1	52 ± 2	43 ± 1	95 ± 2
	2	55 ± 3	41 ± 3	96 ± 2
0.25 M ethanol	1	43 ± 1	38 ± 1	81 ± 2
	2	44 ± 2	36 ± 4	80 ± 3

aqueous phase the pK_a of carboxyl groups of fatty acid is 4.8, hence, at pH 7.0 almost all their molecules are in anionic form. However, if the carboxyl group of fatty acid is situated on the surface of phospholipid bilayer its pK_a is shifted substantially into the alkaline region and reaches 7.2-7.4 [26, 27]. Our calculation gives evidence that the changes in ethanol effect are proportional to changes in the portion of palmitate carboxyl group in anionic form on the mitochondrial membrane surface at pK_a 7.3. It is clear that the higher the ratio between anionic and neutral palmitate forms, the higher portion of them can migrate in the presence of ethanol from one transporter to the other under the influence of their cationic groups, and vice versa, protonation of palmitate anions would result in loss of their capacity to migrate under the action of positive transporter charges.

It is notable that ethanol has no effect on the uncoupling component of palmitate that is insensitive to carboxyatractylate and glutamate. Hence, the migration of palmitate molecules induced by ethanol occurs only within boundaries of ADP/ATP and aspartate/glutamate antiporters and is not spread among other structures involved in the uncoupling. This is only possible if ADP/ATP and aspartate/glutamate antiporters are situated close to each other and are not separated by other proteins that may counteract lateral migration of fatty acids. From the above reasoning, ADP/ATP and aspartate/glutamate antiporters may be considered as a single uncoupling complex. Fatty acid molecules can migrate from one transporter to another (or remain on the spot) within boundaries of this complex depending on the experimental conditions. One can anticipate the existence of at least two different pools of fatty acids participating in uncoupling. The first pool is common for both ADP/ATP and aspartate/glutamate antiporters. Fatty acids of the second pool uncouple oxidative phosphorylation according to other mechanisms considered above.

As pointed out above, ethanol molecules interacting with biological membranes are accumulated mainly on the lipid/water interface between hydrophilic fragments of phospholipid molecules, displacing water [22-24], and fatty acid carboxyl groups are possibly located there [30]. The permittivity of ethanol is substantially lower than that of water [31]. Hence, two opposite charges would attract each other more strongly, and two equal charges would repeal each other more strongly in ethanol compared to water [31]. If water molecules would be replaced by ethanol on the lipid/water interface between hydrophilic fragments of phospholipid molecules, the negatively charged carboxyl groups of fatty acids situated here attract more strongly the positively charged groups of ADP/ATP and aspartate/glutamate antiporters as well as repeal more strongly these proteins on neutralization of their cationic groups respectively by carboxyatractylate and glutamate. This would in turn enhance the ability of fatty acids to migrate from one transporter to the other.

As indicated in our previous studies [15] and confirmed in the present work, addition of 2 mM Mg^{2+} has no influence on stimulation of mitochondrial respiration by palmitate. Ethanol added to mitochondria in the presence of Mg^{2+} has no influence on the ability of fatty acid to stimulate respiration (Fig. 1). Hence, change in the ability of fatty acid to migrate from one transporter to the other is not accompanied by their total uncoupling activity.

Elevation of the $MgCl_2$ concentration from 2 to 8 mM diminishes the uncoupling effect of palmitate. The opinion exists that inhibitory effect of Mg^{2+} is due to the formation of a fatty acid pool inert in uncoupling existing in a form of complexes consisting of poorly water soluble magnesium salt of fatty acid in the incubation medium [11]. A dynamic balance between fatty acid pools participating and not involved in uncoupling would be rapidly achieved under continuous agitation of mitochondrial suspension in a polarograph cell. If the ability of fatty acid anion to interact with cationic groups of phospholipid and proteins would be enhanced by ethanol, this results in shift of equilibrium to the enlargement of fatty acid mitochondrial pool participating in uncoupling. Simultaneously, as the concentration of Mg^{2+} increases, ethanol losses its ability to relieve the recoupling effects of carboxyatractylate or glutamate. Magnesium ions even located at a distance from the membrane surface are able to neutralize the negatively charged groups on the surface [28], including the charged fatty acid carboxyl groups located on mitochondrial membrane surface [32]. It is obvious that neutralization of these groups would result in a loss of fatty acid ability to be translocated by a transporter under the action of their charged groups in one or the other direction.

Thus, one of the ways for regulation of uncoupling effect of fatty acids may be their redistribution between anion transporters involved in uncoupling. For the present only one inducer of such process, namely ethanol, is revealed, and Mg^{2+} is its antagonist. One of the promising directions in investigation on the control of uncoupling effects in fatty acids is the search for other agents that would be able to enhance or diminish fatty acid migration between anion transporters.

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