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Acetoacetate as Regulator of Palmitic Acid-Induced Uncoupling Involving Liver Mitochondrial ADP/ATP Antiporter and Aspartate/Glutamate Antiporter

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Abstract—The effect of acetoacetate on palmitate-induced uncoupling with the involvement of ADP/ATP antiporter and aspartate/glutamate antiporter has been studied in liver mitochondria. The incubation of mitochondria with acetoacetate during succinate oxidation in the presence of rotenone, oligomycin, and EGTA suppresses the accumulation of conjugated dienes. This is considered as a display of antioxidant effect of acetoacetate. Under these conditions, acetoacetate does not influence the respiration of mitochondria in the absence or presence of palmitate but eliminates the ability of carboxyatractylate or aspartate separately to suppress the uncoupling effect of this fatty acid. The action of acetoacetate is eliminated by β-hydroxybutyrate or thiourea, but not by the antioxidant Trolox. In the absence of acetoacetate, the palmitate-induced uncoupling is limited by a stage sensitive to carboxyatractylate (ADP/ATP antiporter) or aspartate (aspartate/glutamate antiporter); in its presence, it is limited by a stage insensitive to the effect of these agents. In the presence of Trolox, ADP suppresses the uncoupling action of palmitate to the same degree as carboxyatractylate. Under these conditions, acetoacetate eliminates the recoupling effects of ADP and aspartate, including their joint action. This effect of acetoacetate is eliminated by β-hydroxybutyrate or thiourea. It is supposed that the stimulating effect of acetoacetate is caused both by increase in the rate of transfer of fatty acid anion from the inner monolayer of the membrane to the outer one, which involves the ADP/ATP antiporter and aspartate/glutamate antiporter, and by elimination of the ability of ADP to inhibit this transport. Under conditions of excessive production of reactive oxygen species in mitochondria at a high membrane potential and in the presence of small amounts of fatty acids, such effect of acetoacetate can be considered as one of the mechanisms of antioxidant protection.

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

Animal mitochondria are not only high-performance energy stations producing ATP and heat, but also have quite a number of other unique functions that allow them to act as a key switch in the cell system of choice between life and death [1-4]. This additional function of mitochondria is largely associated with regulation of the cell redox potential through enhancement or abatement of the production of reactive oxygen species (ROS) by these organelles [1-5]. The respiratory chain is an effective ROS generator in isolated mitochondria. Superoxide anion radical is formed as a result of one-electron reduction of oxygen in complexes I and III. Hydrogen peroxide, hydroxyl radical and other ROS, and reactive nitrogen species can be generated from this anion radical in subsequent chemical (enzymatic and nonenzymatic) reactions [4-9]. ROS hyperproduction results in oxidative damage to mitochondria, which is considered as one of the main factors of cell death [4, 5, 10].

One of the ways of decreasing ROS production in mitochondria is induction of proton conductance of the inner membrane ("mild" uncoupling) by natural uncouplers of oxidative phosphorylation, free long-chain fatty acids [9, 11]. It is obvious that intensification of the protonophore activity of fatty acids contained in mitochondria in small amounts could be a way of antioxidant protection of these organelles under ROS hyperproduction. At present, the mechanisms and pathways of regulating the "mild" uncoupling effect of fatty acids in mitochondria of vitally important organs cannot be considered as fully studied. In liver mitochondria, the protonophoric uncoupling effect of fatty acids involves mainly the protein

Abbreviations: ROS, reactive oxygen species.

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carriers of metabolites of the inner mitochondrial membrane: ADP/ATP antiporter and aspartate/glutamate antiporter [9, 12, 13]. They are supposed to participate in uncoupling by transferring fatty acid anions from the internal monolayer of the membrane to its outer monolayer, while the subsequent transport of undissociated acids through the phospholipid bilayer is performed by the flip-flop mechanism without the involvement of proteins [9, 13]. As we have shown previously, under conditions of antioxidant-induced decrease in lipid peroxidation intensity in liver mitochondria, the protonophoric uncoupling effect of palmitate is substantially suppressed by physiological substrates of the ADP/ATP antiporter and aspartate/glutamate antiporter: ADP, aspartate, and glutamate [14-16]. Under these conditions, the development of oxidative stress induced by endogenous processes in the mitochondria of old rats or by the oxidative agent *tert*-butylhydroperoxide results in considerable intensification of the protonophoric activity of this fatty acid [14-16]. It is known that passive proton leakage through the inner membrane in liver mitochondria can intensify in the case of ADP/ATP antiporter modification by one of the final products of lipid peroxidation, 4-hydroxynonenal [5, 8, 17]. However, mechanism of regulation of fatty acid protonophoric activity, in our opinion, is rather "stiff", i.e. non-physiological, due to the cytotoxicity of 4-hydroxynonenal [18-20] and its ability to covalently modify different mitochondrial proteins, reducing their functional activity [20-22]. It would be interesting to determine whether the protonophoric activity of fatty acids in liver mitochondria can increase under "milder" conditions, i.e. without intensification of lipid peroxidation. Acetoacetate has drawn our attention as a potential "mild" modifier of the protonophoric activity of fatty acids.

Acetoacetate is synthesized in liver mitochondria from acetyl-CoA, the main source of which is β-oxidation of fatty acids [23, 24]. The increase in the level of free fatty acids in an organism under intensive physical load, starvation, or in a pathological state such as diabetes results in considerable intensification of production of acetoacetate and β-hydroxybutyrate formed through its reduction [24, 25]. In contrast to 4-hydroxynonenal, acetoacetate not only has a cytotoxic effect but also contributes to survival and maintenance of the functional activity of various cells [26, 27]. This protective effect of acetoacetate is supposedly associated with its antioxidant effect [27, 28]. It is known that incubation of liver mitochondria with acetoacetate in the presence of succinate and rotenone results in oxidation of mitochondrial pyridine nucleotides (mainly NADH) but not sulfhydryl groups of glutathione [29-31]. This effect of acetoacetate can lead to oxidation of SH groups of some mitochondrial proteins [29], including ADP/ATP antiporter, and such modification of this carrier is accompanied by increase in the rate of ADP transport across the inner membrane [31].

The goal of the present work was to elucidate the role of acetoacetate as a regulator of the protonophoric uncoupling activity of fatty acids with the participation of ADP/ATP antiporter and aspartate/glutamate antiporter in liver mitochondria under suppression of lipid peroxidation. Toward this goal, we have studied the influence of ligands of these carriers (carboxyatractylate, ADP, and aspartate), a reducer of pyridine nucleotides (β-hydroxybutyrate), a reducer of thiol groups (thiourea), and antioxidant (Trolox) on palmitate-induced respiration in the absence and presence of acetoacetate.

MATERIALS AND METHODS

Mitochondria from the liver of mature male white rats weighing 220-250 g were isolated by a standard method of differential centrifugation followed by liberation from endogenous fatty acids using fatty acid-free BSA as described previously [12]. The isolation medium contained: 250 mM sucrose, 1 mM EGTA, and 5 mM Mops-Tris, pH 7.4. Mitochondrial protein concentration was measured by the biuret method with BSA as the standard. During the experiment, mitochondrial suspension (70-80 mg mitochondrial protein in 1 ml) was kept on ice in a narrow test tube (Eppendorf, Germany). Mitochondrial respiration was recorded at 25°C using a Clark oxygen electrode and a LP-9 polarograph. Mitochondrial protein concentration in the oxygen-measuring cell was 0.9-1.1 mg/ml. The incubation medium contained: 200 mM sucrose, 20 mM KCl, 5 mM succinic acid, 2 mM $MgCl_2$, 0.5 mM EGTA, and 5 mM Mops-Tris, pH 7.0. Oligomycin (2 μg/ml) and rotenone (2 μM) were added into the oxygen-measuring cell immediately after the mitochondria. The respiration rate during oxidative ATP synthesis (state 3) was measured in the incubation medium containing additionally 5 mM KH_2PO_4 without oligomycin. In this case, 200 μM ADP was added to the mitochondria 2 min after the addition of rotenone. The ADP/O coefficient was determined by the pulse method [32]. The recoupling effects of carboxyatractylate and aspartate were expressed as percentage and defined as ratio of respiration inhibition in the presence of palmitate by one of these recoupling agents to respiration stimulation by palmitate according to the formula $100 \cdot \Delta J_u/(J_u - J_o)$, where J_u and J_o were respiration rates in the presence and absence of palmitate, respectively, and ΔJ_u was respiration rate reduction by a recoupling agent. The content of conjugated dienes in the mitochondria was determined after extraction in heptane by spectrophotometry as described [14]. The increase in the content of conjugated dienes in the mitochondria was expressed in relative units as factor α determined by the formula: $\alpha = (\Delta A - \Delta A_0)/\Delta A_0$, where ΔA_0 and ΔA were the optical densities of heptane extract at 233 nm in the initial moment of incubation of the mitochondria and after 5 min, respectively.

The mean value of ΔA_0 was 0.073 ± 0.003 OD units per mg protein (n = 9).

Mops, Tris, palmitic acid, oligomycin, succinic acid, potassium aspartate, sodium β-hydroxybutyrate, carboxyatractylate, and BSA (fatty acid-free) (Sigma, USA), rotenone, ADP, and EGTA (Serva, Germany), 2,4-dinitrophenol, lithium acetoacetate, and sucrose (Fluka, Germany), Trolox (Aldrich, Germany), KCl and $MgCl_2$ (Merck, Germany) were used in the work. Other reagents of chemical purity and high-purity grade were produced in Russia. Palmitic acid solution (20 mM) in twice distilled ethanol was used.

RESULTS AND DISCUSSION

As we have shown previously [33] and confirmed in the present work (Table 1), the incubation of mitochondria in a controlled state (in the absence of ADP or uncouplers) in the presence of succinate, rotenone, oligomycin, and EGTA is accompanied by the accumulation of conjugated dienes. The accumulation of lipid peroxidation products, including conjugated dienes, is an indicator of intensified generation of ROS and, in particular, superoxide anion radical [34, 35]. Acetoacetate retards the accumulation of conjugated dienes in the controlled state but does not influence the respiration in state 3, the respiratory control coefficient, and the efficiency of oxidative phosphorylation of liver mitochondria (Table 1). Consequently, acetoacetate does not intensify but even markedly weakens lipid peroxidation; therefore, this effect does not result in accumulation of the final products of lipid peroxidation—reactive aldehydes including

Table 1. Comparison of variations in the content of conjugated dienes in liver mitochondria in the controlled state (α, relative units), mitochondrial respiration rate in state 3 (J_3, nmol O_2/min per mg protein), ADP/O (relative units), and respiration control (RCR, relative units) coefficients in the absence and presence of acetoacetate

Index	Control	Acetoacetate, 5 mM
α	0.196 ± 0.012	0.128 ± 0.013*
J_3	51.0 ± 3.8	58.6 ± 4.1
ADP/O	1.78 ± 0.02	1.74 ± 0.03
RCR	4.17 ± 0.10	4.64 ± 0.21

Note: Experimental conditions and incubation medium composition are described in "Material and Methods". Here and in Table 2, mean values ± standard error of mean (n = 4, 5) are given.

* Difference between experiment (with acetoacetate) and control (without acetoacetate) are statistically reliable, $p < 0.01$ (Student's criterion).

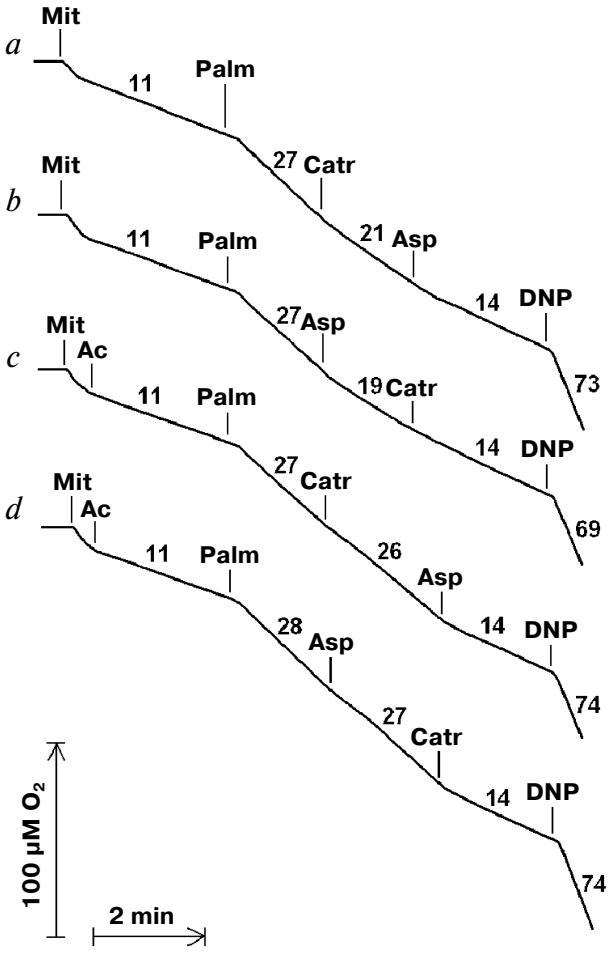

Fig. 1. Stimulation of mitochondrial respiration by palmitate and the effects of aspartate and carboxyatractylate on respiration in the absence (*a*, *b*) and presence (*c*, *d*) of acetoacetate. Experimental conditions and incubation medium composition are described in the "Materials and Methods". Mit, mitochondria (0.9 mg protein/ml); Ac, acetoacetate (5 mM); Palm, palmitate (30 μM); Asp, aspartate (3 mM); Catr, carboxyatractylate (1 μM); DNP, 2,4-dinitrophenol (50 μM).

4-hydroxynonenal. These results, being evidence of the antioxidant properties of acetoacetate, are in good agreement with known literature data obtained under different experimental conditions and from different biological objects [27, 28, 36].

In the experiments, palmitate was used as the anion of one of the most widespread natural fatty acids [37]. As shown in previous studies [14], the dependence of respiration rate of liver mitochondria on palmitate concentration of 0 to 40 μM is nearly linear. In the present work, it has been ascertained that acetoacetate (5 mM) does not influence the character of this dependence (data not shown). In all subsequent experiments, palmitate concentration was 30 μM. In the presence of EGTA, magnesium ions, rotenone, and oligomycin the protonophoric activity of fatty acids, as substantiated previously [38, 39],

can be judged by the stimulation of mitochondrial respiration. Under these conditions, as shown in Fig. 1, palmitate-induced respiration of liver mitochondria in the absence of acetoacetate is suppressed on subsequent addition of carboxyatractylate and then aspartate (curve *a*), or first aspartate and then carboxyatractylate (curve *b*). Under incubation of the mitochondria with acetoacetate, respiration in the presence of palmitate is not suppressed by aspartate or carboxyatractylate added after palmitate; however, these agents considerably inhibit respiration when added in another succession (carboxyatractylate after aspartate or aspartate after carboxyatractylate) (Fig. 1, curves *c* and *d*). However, acetoacetate has no effect on respiration of mitochondria in the controlled state (without palmitate), in the presence of palmitate only, or with palmitate in the presence of aspartate and carboxyatractylate simultaneously (Fig. 1). It should be noted that this change in the palmitate-induced uncoupling effect by acetoacetate is not associated with the induction of calcium-dependent nonspecific permeability of the inner membrane (pore opening). This is favored by the following arguments: first, the effect of acetoacetate is observed in the presence of a chelating agent for calcium ions (EGTA); second, carboxyatractylate in combination with aspartate inhibits palmitate-stimulated respiration in the presence of acetoacetate (Fig. 1, curve *d*) but, in contrast, intensifies respiration under pore opening [13].

The ability of carboxyatractylate or aspartate to suppress the uncoupling effect of fatty acids can be expressed quantitatively as a recoupling effect [38, 39]. In the absence of influence on uncoupling (as, e.g. in Fig. 1, curves *c* and *d*), the recoupling effect is equal to 0; under total palmitate-induced suppression of stimulation of respiration, the recoupling effect reaches its maximum (100%). As follows from Table 2, the incubation of mitochondria in the controlled state with acetoacetate, according to the conditions in Fig. 1, results in change in the nature of recoupling effects of aspartate and carboxyatractylate. Each of these agents separately does not influence the action of palmitate, while in the presence of both agents the uncoupling effect of palmitate is reduced by 77%. In subsequent experiments, mitochondria were incubated in the controlled state in the presence of acetoacetate and one of the following compounds: β-hydroxybutyrate, Trolox, or thiourea. As is known, β-hydroxybutyrate is a reducer of mitochondrial pyridine nucleotides and prevents the oxidative effect of acetoacetate [31, 36]; thiourea is known as an effective reducer of thiol groups [40]; and Trolox, a water-soluble analog of α-tocopherol, is an effective eliminator of free radicals [41]. As seen from Table 2, β-hydroxybutyrate and thiourea, being added together with acetoacetate, reduce the recoupling effects of aspartate and carboxyatractylate, whereas the antioxidant Trolox has no such effect. Under these conditions, as we have shown previously [33] and confirmed in the present work (data not shown), Trolox substantially suppresses the accumulation of conjugated dienes in mitochondria, i.e. has a marked antioxidant effect.

The results demonstrate that incubation of liver mitochondria with acetoacetate changes the nature of uncoupling effect of fatty acids to the same extent as we have observed previously under oxidative stress induced by endogenous processes in the mitochondria of old rats or by the action of the oxidative agent *tert*-butylhydroperoxide [14, 16, 33]. However, in contrast to the above, the modulating effect of acetoacetate is not associated with the development of oxidative stress because it is not accompanied by intensification of lipid peroxidation and not eliminated by the antioxidant Trolox. The fact that the modifying effect of acetoacetate is not observed in the presence of β-hydroxybutyrate and thiourea suggests that its action is caused by oxidation of pyridine nucleotides

Table 2. Influence of acetoacetate in the absence and presence of Trolox, β-hydroxybutyrate, and thiourea on recoupling effects of aspartate (Asp) and carboxyatractylate (Catr) under palmitate-induced uncoupling effect in liver mitochondria

Experimental conditions	Recoupling effect, %		
	Asp	Catr	Asp + Catr
Control (n = 5)	46.3 ± 1.1	32.5 ± 1.0	79.0 ± 1.1
Acetoacetate (n = 5)	4.6 ± 1.6	2.1 ± 1.2	77.2 ± 1.7
Acetoacetate + Trolox (n = 3)	5.8 ± 2.4	3.9 ± 2.8	79.4 ± 2.9
Acetoacetate + β-hydroxybutyrate (n = 4)	46.6 ± 0.8	32.8 ± 0.4	79.5 ± 1.1
Acetoacetate + thiourea (n = 4)	45.4 ± 0.6	33.6 ± 1.1	79.1 ± 1.5

Note: Experimental conditions and incubation medium composition are described in "Materials and Methods". Asp, aspartate (3 mM); Catr, carboxyatractylate (1 μM). Acetoacetate, aspartate, and carboxyatractylate are added as in Fig. 1. Trolox (30 μM), or β-hydroxybutyrate (10 mM), or thiourea (0.3 mM) are added together with acetoacetate.

and critical SH groups, which probably belong to the ADP/ATP antiporter and aspartate/glutamate antiporter that have SH groups subject to oxidation [42, 43].

As already mentioned in the introduction, the mechanism of protonophoric uncoupling by fatty acids consists of at least two subsequent stages, and the recoupling effect of carboxyatractylate or aspartate is realized only at one of them [9, 13]. It would be interesting to assume that the stage limiting this process in mitochondria is changed under the influence of acetoacetate. It should be noted that electron transport in the respiratory chain in all cases does not limit the uncoupling, because the maximum rate of mitochondrial respiration measured in the presence of 2,4-dinitrophenol in the optimal concentration is much higher than the rate during the uncoupling studies (Fig. 1). It is known that the limiting stages of energy transduction in mitochondria can be revealed by means of respective specific enzyme inhibitors by analyzing the resultant curves of inhibitory titration [44, 45]. Previously we demonstrated that the curves of titration of mitochondrial respiration by carboxyatractylate and aspartate in the presence of palmitate-induced uncoupling effect were of typical shape, showing the response to the first additions of these recoupling agents [39].

Analogous results have been obtained in the present study during titration of respiration by carboxyatractylate in the presence of aspartate (Fig. 2a, curve *1*) or by aspartate in the presence of carboxyatractylate (Fig. 2b, curve *1*). These data demonstrate that the palmitate-induced process of uncoupling is limited at the stages with an effect of carboxyatractylate (ADP/ATP-antiporter) or aspartate (aspartate/glutamate antiporter). In accordance with the cyclic mechanism of the uncoupling effect of fatty acids [9, 13], this limiting stage is the transfer of fatty acid anion from the inner monolayer of the membrane to its outer monolayer with the participation of these carriers. Subject to the incubation of mitochondria in the controlled state with acetoacetate, the curves of titration by carboxyatractylate in the presence of aspartate (Fig. 2a, curve *2*) or by aspartate in the presence of carboxyatractylate (Fig. 2b, curve *2*) are S-shaped, i.e. the recoupling agents in low concentrations inhibit respiration to a lesser extent compared to their subsequent additions in the same concentrations.

These results indicate that palmitate-induced uncoupling during the incubation of mitochondria with acetoacetate is limited by the stage where the effect of carboxyatractylate or aspartate is absent. In this case, the limiting stage, in accordance with the cyclic mechanism of the uncoupling effect of fatty acids [9, 13], is transfer of the undissociated acid across the bilayer from the outer monolayer of the membrane to its inner monolayer by the flip-flop mechanism without the involvement of proteins. We found analogous results previously under oxidative stress induced by endogenous processes in the mitochondria of old animals or by the effect of the oxidative agent *tert*-butylhydroperoxide [14, 16]. However, as mentioned above, the effect of acetoacetate considered in the present work is not associated with the development of oxidative stress.

It can be supposed that as a consequence of acetoacetate-induced oxidative modification of the ADP/ATP

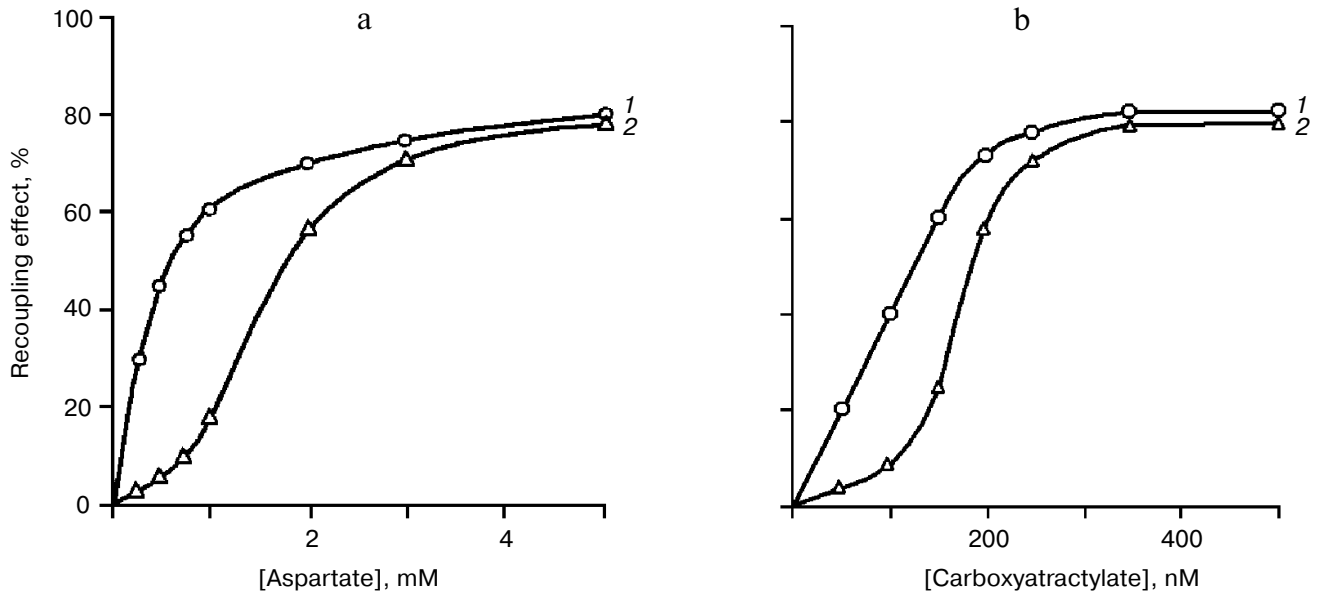

Fig. 2. Concentration dependence of recoupling effects of aspartate in the presence of carboxyatractylate (a) and carboxyatractylate in the presence of aspartate (b) under palmitate-induced uncoupling in rat liver mitochondria in the absence (*1*) and presence (*2*) of acetoacetate (5 mM). Additions are the same as in the legend to Fig. 1.

antiporter and aspartate/glutamate antiporter these carriers become able to transport fatty acid anions from the inner to outer side of the membrane at a higher rate. As a result, as shown above, the limiting stage of uncoupling will be not the transport of a fatty acid anion (the stage sensitive to carboxyatractylate or aspartate) but the transfer of a protonated fatty acid molecule from the outer monolayer of the membrane to its inner monolayer (the stage insensitive to carboxyatractylate or aspartate). In this case, exclusion of one of the carriers from the uncoupling process will not be accompanied by the decrease in palmitate uncoupling activity due to greater activity of the other carrier.

In further experiments (Fig. 3), carboxyatractylate was replaced by ADP, which is a physiological substrate of the ADP/ATP antiporter. ADP concentration was chosen on the basis of known data on its content in liver cells [46, 47]. It was shown previously that ADP suppresses the uncoupling effect of palmitate to the same extent as carboxyatractylate [48]. Recently we established that ADP does not influence the uncoupling effect of palmitate during succinate oxidation in the presence of rotenone and oligomycin during development of oxidative stress in liver mitochondria [14-16]. Under the same conditions but in the presence of antioxidants or reducers of pyridine nucleotides, ADP suppresses the uncoupling effect of palmitate to the same extent as carboxyatractylate [14-16]. As shown in Fig. 3 (curve *a*), ADP does not influence palmitate-stimulated mitochondrial respiration in the absence of Trolox but effectively suppresses it in the presence of this antioxidant (Fig. 3, curve *b*). Therefore, for the recoupling effect of ADP to be displayed, the free-radical and peroxidation processes in mitochondria must by inhibited. Aspartate has a recoupling effect both in the presence and in the absence of this antioxidant (Fig. 3, curves *a* and *b*). During the incubation of mitochondria with Trolox and acetoacetate simultaneously, ADP and aspartate have no effect on the uncoupling activity of palmitate (Fig. 3, curve *c*). Under the influence of acetoacetate, the respiration rate of the liver mitochondria in the presence of palmitate, ADP, and aspartate increased by 79% (see curves *b* and *c* in Fig. 3), while the protonophoric activity of palmitate, defined as the value of respiration stimulation by the fatty acid [38, 39], increased from 3 to 14 nmol O_2/min per mg protein, i.e. nearly 5-fold. The addition of β-hydroxybutyrate to mitochondria together with Trolox and acetoacetate results in recovery of the ability of ADP and aspartate to suppress the uncoupling effect of palmitate (Fig. 3, curve *d*). An analogous effect (like that of β-hydroxybutyrate) is produced by thiourea (data not shown).

The results demonstrate that acetoacetate eliminates the ability of ADP and aspartate to suppress the uncoupling effect of palmitate under Trolox-induced inhibition of free-radical and peroxidation processes in the mitochondria. We consider such effect of acetoacetate as

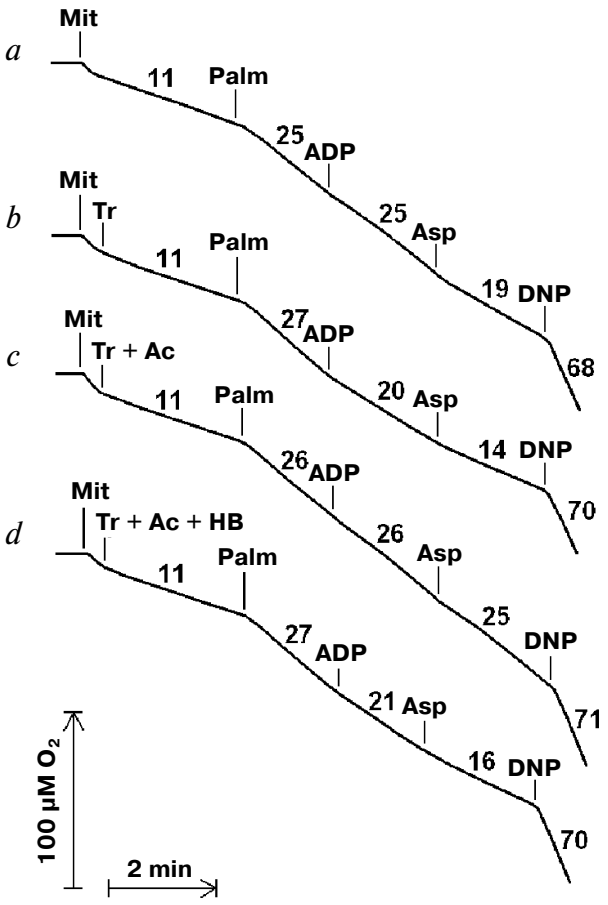

Fig. 3. Stimulation of mitochondrial respiration by palmitate and influence of ADP and aspartate on respiration in the absence (*a*) and presence of Trolox (*b*), Trolox and acetoacetate (*c*), and Trolox, acetoacetate, and β-hydroxybutyrate (*d*). Tr, Trolox (30 μM); HB, β-hydroxybutyrate (10 mM); ADP, ADP (200 μM). Other additions were the same as in the legend to Fig. 1.

intensification of the protonophoric uncoupling activity of palmitate in the presence of these physiological substrates of the ADP/ATP antiporter and aspartate/glutamate antiporter. Previously it was already supposed that the ADP/ATP antiporter, being mainly in the *m*-conformation, in the presence of ADP can transfer fatty acid anion from the inner monolayer of the membrane to its outer monolayer only given its oxidative modification [14]. The data of the present work are in good agreement with this supposition.

Thus, incubation of liver mitochondria with acetoacetate in the presence of physiological substrates of the ADP/ATP antiporter and aspartate/glutamate antiporter (ADP and aspartate, respectively) intensifies the protonophoric uncoupling activity of palmitate. This can be caused both by increase in the rate of fatty acid anion transfer from the inner monolayer of the membrane to its outer monolayer with the participation of ADP/ATP antiporter and aspartate/glutamate antiporter and by

elimination of the ability of ADP to inhibit this process. Previously, analogous changes in the nature of the uncoupling effect of palmitate were observed in liver mitochondria under oxidative stress induced by endogenous processes in mitochondria of old rats or under the action of the oxidative agent *tert*-butylhydroperoxide [14, 16, 33]. However, in contrast to the above, the effect of acetoacetate is exhibited even with the lowering activities of free-radical and peroxidation reactions and, therefore, is not associated with the effects of lipid peroxidation products: 4-hydroxynonenal and other aldehydes. It was mentioned above that the studied effect of acetoacetate can be associated with the oxidation of pyridine nucleotides and critical SH groups of mitochondria. SH groups susceptible to oxidation are present both in the ADP/ATP antiporter [42] and in the aspartate/glutamate antiporter [43]. In the mitochondrial matrix, there is a connection between the redox state of pyridine nucleotides and the thiol groups of mitochondrial proteins, which might not involve glutathione [29, 31]. The results suggest that acetoacetate is one of the regulators of the protonophoric uncoupling effect of fatty acids with the participation of ADP/ATP antiporter and aspartate/glutamate antiporter in liver mitochondria. Under excess ROS production by mitochondria at high membrane potential and in the presence of small quantities of fatty acids, intensification of the protonophoric activity of these metabolites by acetoacetate can be considered as a mechanism of its antioxidant effect.

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