Biochemistry (Moscow), Vol. 68, No. 6, 2003, pp. 618-626. Translated from Biokhimiya, Vol. 68, No. 6, 2003, pp. 759-768.

Temperature Dependence of Rat Liver Mitochondrial Respiration with Uncoupling of Oxidative Phosphorylation by Fatty Acids. Influence of Inorganic Phosphate

V. N. Samartsev*, S. A. Chezganova, L. S. Polishchuk, A. P. Paydyganov, O. V. Vidyakina, and I. P. Zeldi

Mari State University, pl. Lenina 1, Yoshkar-Ola 421001, Russia; fax: (836-2) 45-4581; E-mail: samartsev@marsu.ru

Received March 1, 2002
Revision received April 29, 2002

Abstract—The respiration rate of liver mitochondria in the course of succinate oxidation depends on temperature in the presence of palmitate more strongly than in its absence (in state 4). In the Arrhenius plot, the temperature dependence of the palmitate-induced stimulation of respiration has a bend at 22°C which is characterized by transition of the activation energy from 120 to 60 kJ/mol. However, a similar dependence of respiration in state 4 is linear over the whole temperature range and corresponds to the activation energy of 17 kJ/mol. Phosphate partially inhibits the uncoupling effect of palmitate. This effect of phosphate is increased on decrease in temperature. In the presence of phosphate the temperature dependence in the Arrhenius plot also has a bend at 22°C, and the activation energy increases from 128 to 208 kJ/mol in the range from 13 to 22°C and from 56 to 67 kJ/mol in the range from 22 to 37°C. Mersalyl (10 nmol/mg protein), an inhibitor of the phosphate carrier, similarly to phosphate, suppresses the uncoupling effect of laurate, and the effects of mersalyl and phosphate are not additive. The recoupling effects of phosphate and mersalyl seem to show involvement of the phosphate carrier in the uncoupling effect of fatty acids in liver mitochondria. Possible mechanisms of involvement of the phosphate carrier in the uncoupling effect of fatty acids are discussed.

Key words: liver mitochondria, free oxidation, respiration, uncoupling, fatty acids, temperature dependence, Arrhenius plot, phosphate carrier

About 40% of oxygen consumption by mitochondria in liver cells is not associated with synthesis of ATP [1, 2]. This respiration, also called free oxidation [3, 4], is suggested to be physiologically important [2-6].

Free oxidation in mitochondria can be due to various processes in the inner membrane, in particular, to the cyclic transport of potassium ions which is provided by electrophoretic entrance of K^+ into the matrix and its subsequent exit by means of K^+/H^+-exchange [4, 7], and to uncoupling proteins UCP-2 and UCP-3, which are analogs of UCP-1 [6, 8]. However, the effect of free fatty acids, the level of which in animal and human cells increases in various physiological and pathological states, is thought to be most important [3-5, 9, 10].

Mechanisms of the uncoupling effect of fatty acids in mitochondria are now intensively studied, and certain progress has been achieved (reviews [4, 5, 11-13]). The protonophore uncoupling effect of fatty acids in liver mitochondria is found to occur with involvement of the ADP/ATP-antiporter [14], aspartate/glutamate antiporter [15], and the dicarboxylate carrier [16]. The first two carriers mainly contribute to the uncoupling: they are responsible for about 80% of the uncoupling effect of fatty acids [17, 18]. In a reconstituted system with the phosphate carrier from yeast mitochondria incorporated into liposomes this protein was recently shown to accelerate the transmembrane cyclic transport of protons by fatty acids as well as in the system with the uncoupling protein UCP-1 of mitochondria from brown fat tissue [19, 20]. Fatty acids also inhibit the electroneutral capture of phosphate by liver and heart mitochondria [20].

Abbreviations: DNP) 2,4-dinitrophenol; E_a) activation energy; TTFB) tetrachlorotrifluoromethylbenzimidazole; FCCP) carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone; UCP-1) uncoupling protein of brown fat tissue mitochondria; UCP-2 and UCP-3) uncoupling protein analogs of UCP-1; $\Delta\mu_{H^+}$) difference of electrochemical potential of protons on the inner mitochondrial membrane; $\Delta\Psi$) difference of electric potential on the inner mitochondrial membrane.

* To whom correspondence should be addressed.

0006-2979/03/6806-0618$25.00 ©2003 MAIK "Nauka/Interperiodica"

Probably, the phosphate carrier is also involved in the uncoupling effect of fatty acids in liver mitochondria. In this case phosphate, a substrate of this carrier, is also expected to have a recoupling effect similarly to substrates of other anion carriers involved in the uncoupling [14-16].

According to a hypothesis of V. P. Skulachev [21] which underlies current concepts on the mechanism of the uncoupling effect of fatty acids, the transfer of fatty acid anions from the internal membrane layer to the external one is a function of the UCP-1 and ADP/ATP-antiporter, and undissociated acids are subsequently transferred across the bilayer by the flip-flop mechanism without involvement of proteins. This hypothesis was further developed and experimentally confirmed by works on a reconstructed system with the UCP-1 incorporated into liposomes [22]. This hypothesis is varied to explain the uncoupling effect of fatty acids with involvement of the ADP/ATP- and aspartate/glutamate antiporters [4, 5, 11-13, 18]. Fatty acid anions pass very slowly from the internal membrane monolayer onto the external one because of a very high energy barrier [22, 23]. Positively charged groups of membrane proteins or lipophilic anions can promote the overcoming of this barrier [22, 23]. The activation energy of the transport of fatty acid anions can be determined from the temperature dependence of the uncoupling effect of fatty acids, because just the transport of fatty acid anions limits this process [4, 5, 11, 12, 21, 22].

To elucidate mechanisms and pathways of regulation of free oxidation in mitochondria, in the present work the temperature dependence of respiration in the case of partial uncoupling by fatty acids was studied, as well as the influence of phosphate on this dependence. The finding of a significant recoupling effect of phosphate and also of mersalyl, which inhibits the phosphate transport at a relatively low temperature, suggests that the phosphate carrier is involved in the uncoupling effect of fatty acids in liver mitochondria.

MATERIALS AND METHODS

Mitochondria were isolated from livers of 180-200 g white rats by differential centrifugation. To remove endogenous fatty acids, the mitochondria were preincubated with BSA made fatty acid-free as described in [24]. The isolation medium contained 250 mM sucrose, 2 mM EGTA, and 5 mM Mops-Tris (pH 7.4). The suspension of mitochondria (60-70 mg protein per 1 ml) was stored on ice. Protein content was determined by the biuret method with BSA as the standard.

Respiration of mitochondria was recorded with a Clark type oxygen electrode or with an open platinum electrode in a thermostatted cell. The incubation medium contained 250 mM sucrose, 5 mM potassium succinate, 10 mM KCl, 0.5 mM EGTA, 2 mM $MgCl_2$, and 5 mM Mops-Tris (pH 7.4). To study the effect of phosphate, the incubation medium was additionally supplemented with 5 mM KH_2PO_4 (pH 7.4). Immediately on addition of mitochondria (1 mg/ml), oligomycin (2 μg/ml) and 2 μM rotenone were added into the cell. Preparations of mitochondria were used only with the respiratory control quotient of no less than 7 that was measured as the stimulation degree of respiration by 50 μM DNP at 25°C.

The uncoupling effect of fatty acids was determined by the stimulation value of mitochondrial respiration ($J_u - J_o$) or by the stimulation degree of respiration (by the formula $(J_u - J_o)/J_o$, where J_o and J_u are the rates of mitochondrial respiration before and after the addition of a fatty acid, respectively.

The recoupling effect of phosphate was calculated by the formula $(J_u - J_{up})/(J_u - J_o)$, where J_{up} is the respiration rate in the presence of phosphate and fatty acid.

The energy activation (E_a) was determined by the plot based on the integral form of the Arrhenius equation [25].

Mops, Tris, ADP, palmitic and lauric acids, oligomycin, mersalyl, potassium succinate, and BSA purified of fatty acids were from Sigma (USA); rotenone and EGTA were from Serva (Germany); KCl, KH_2PO_4, and $MgCl_2$ were from Merck (Germany).

Sucrose was recrystallized by precipitation with ethanol from aqueous solution. Solutions of palmitic and lauric acids in ethanol (10 and 20 mM) were used.

RESULTS

The temperature dependence of mitochondrial respiration was studied in the presence of EGTA, magnesium ions, and oligomycin in the incubation medium. By binding calcium ions, EGTA prevents the induction by fatty acids of Ca^{2+}-dependent nonspecific permeability of mitochondria [26, 27], whereas magnesium ions suppress the ionophore effect of fatty acids [28]. The presence of oligomycin is necessary to inhibit cycles of ADP hydrolysis-resynthesis, one of which is known to be induced by fatty acids [29]. We have earlier shown [18] that in the presence of EGTA, magnesium ions, and oligomycin the mitochondrial respiration is stimulated by fatty acids only due to their protonophore effect, mainly with involvement of the ADP/ATP- and aspartate/glutamate antiporters. It should be noted that the mitochondria contained no fatty acids, because their respiration in state 4 was not changed on addition into the incubation medium of BSA free of fatty acids (data not presented).

As the temperature was increased, the respiration rate increased in state 4 and also in the presence of palmitate (Fig. 1). However, with increase in temperature from 13 to 37°C the respiration rate in state 4

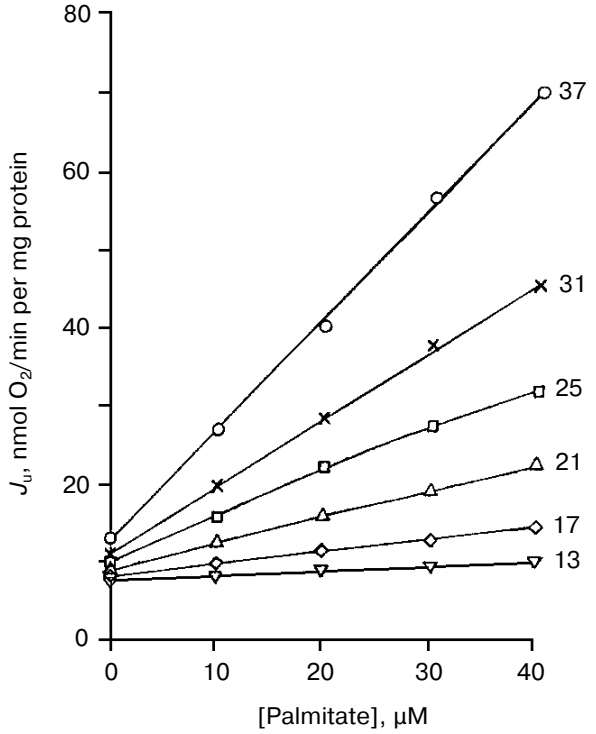

Fig. 1. Stimulation of mitochondrial respiration by palmitate at 13, 17, 21, 25, 31, and 37°C. The experiment conditions are described in "Materials and Methods".

increased only 1.7-fold, whereas in the presence of 40 μM palmitate it increased 6.6-fold. A subsequent addition of DNP at every temperature resulted in an additional 2-3-fold stimulation of respiration (data not presented). Thus, palmitate at the concentration of 40 μM had only a partial uncoupling effect on mitochondria, i.e., the transport of a form of palmitate limited the process. Note that at every temperature the dependence of the respiration rate on the palmitate concentration was close to linear. And the close to linear dependence of the respiration rate of mitochondria on the protonophore concentration suggests that the stage of proton reentry into the matrix should mainly contribute to the control of respiration [30, 31].

The stimulation value of respiration is proposed as a parameter of the specific uncoupling effect of palmitate. This value is determined as the difference between the rate of mitochondria respiration before and after the addition of this fatty acid. This is reasoned by the finding that at the fatty acid concentrations used the respiration rate in state 4 is a component of the respiration rate in the presence of palmitate. According to our calculations (based on data of [32-36]), a little decrease in $\Delta\Psi$ along with a 2-3-fold activation of respiration by palmitate decreased this component only by 5-10%.

In the Arrhenius plot the temperature dependence of the palmitate-induced stimulation of mitochondrial respiration displays two linear regions with a bend at 22°C (Fig. 2). By contrast, the temperature dependence of mitochondrial respiration in state 4 has no bend in the Arrhenius plot (Fig. 3). When the respiration rate is used as a parameter of the uncoupling effect of palmitate without correction to state 4 at the palmitate concentrations of 10 and 20 μM, the temperature dependence in the Arrhenius plot is a straight line and the curve has no bend (data not presented). Only at palmitate concentrations of 30 and 40 μM do two linear regions appear in this plot with a bend at 22°C (data not presented). The temperature dependences of the respiration stimulation with 5 μM DNP (the respiration increased 2.6-fold) and at the complete uncoupling with DNP in the Arrhenius plot are characterized by two linear regions with a bend at 22°C (data not presented).

Values of E_a for different states of mitochondria are presented in Table 1. The table shows that the mitochondrial respiration in state 4 is characterized by a low value of E_a, while the uncoupling effect of palmitate is charac-

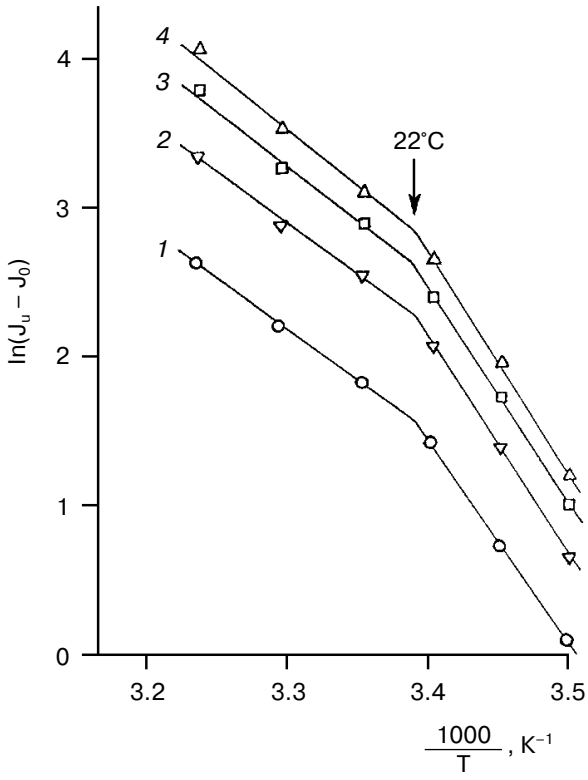

Fig. 2. Arrhenius plot of the temperature dependence of stimulation of mitochondrial respiration (in nmol O_2/min per mg protein) by palmitate at the concentrations of 10 (*1*), 20 (*2*), 30 (*3*), and 40 μM (*4*).

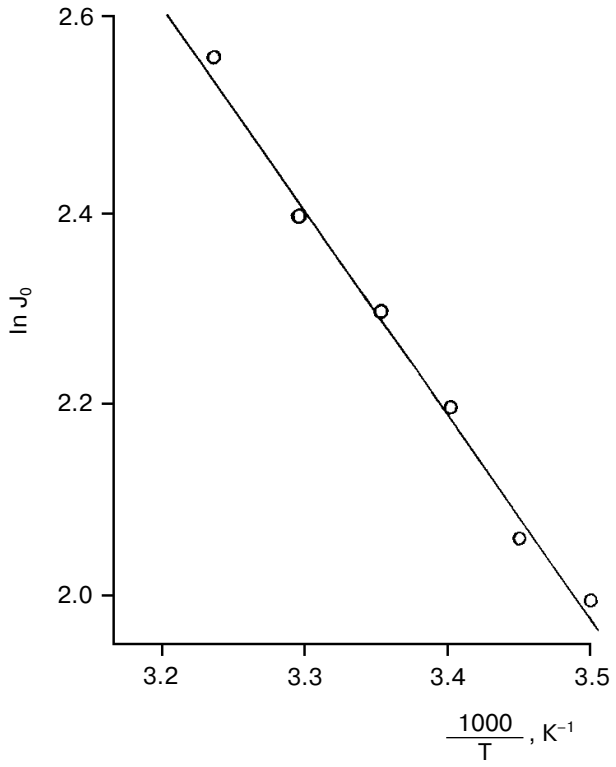

Fig. 3. Arrhenius plot of the temperature dependence of mitochondrial respiration (in nmol O_2/min per mg protein) in state 4.

terized by the some times greater value of E_a. This value does not depend on the concentration of palmitate if the uncoupling effect has been determined by the stimulation value of respiration (Table 1). Note that the temperature dependence of the uncoupling effect of DNP is characterized by similar values of E_a, and lower values of E_a for the mitochondrial respiration were found on complete uncoupling by DNP (Table 1).

At 25°C phosphate suppressed slightly the uncoupling effect of palmitate (Fig. 4a), but its effect was significantly increased on decrease in temperature to 13°C (Fig. 4b). An increase in the palmitate concentration at every temperature decreased the recoupling effect of phosphate (Fig. 5). In the Arrhenius plot, the temperature dependence of the palmitate-induced stimulation of respiration in the presence of 5 mM phosphate presents two linear regions with a bend at 22°C (Fig. 6), similarly to the case of phosphate absence. However, the inclination of this dependence in the presence of phosphate is significantly greater than in its absence (Fig. 6). In the temperature range from 13 to 22°C the E_a value under the influence of phosphate increases from 128 to 208 kJ/mol, and in the range from 22 to 37°C it increases from 56 to 67 kJ/mol. These findings show that phosphate significantly increases the temperature dependence of the uncoupling effect of palmitate. Note, that at every temperature phosphate failed to significantly influence both the mitochondrial respiration in state 4 and the maximal mitochondrial respiration on complete uncoupling by DNP (data not presented).

At the standard temperature of 25°C, the uncoupling effect of fatty acids was about 80% suppressed on the combined addition of carboxyatractylate and glutamate (or aspartate), and this suggested that both the ADP/ATP- and aspartate/glutamate antiporters should contribute to this process [15, 17, 18]. It was shown above that the recoupling effect of phosphate at this temperature was insignificant. It was interesting to find out how the contribution of these antiporters to the uncoupling effect of palmitate would change with decrease in temperature when the recoupling effect of phosphate was stronger. Table 2 shows that at 19°C phosphate suppressed

Table 1. Bend point in the Arrhenius plot (T_m) and the activation energy (E_a) observed for the stimulation of mitochondrial respiration with palmitate and DNP. Mean values ± error of the mean are presented (n = 3-4)

Palmitate, μM	DNP, μM	T_m, °C	E_a, kJ/mol	
			(13-22°C)	(22-37°C)
0	—	none	17 ± 2	17 ± 2
10	—	22 ± 1	112 ± 12	57 ± 4
20	—	22 ± 0	128 ± 6	56 ± 3
30	—	22 ± 1	121 ± 11	60 ± 5
40	—	22 ± 2	121 ± 15	60 ± 6
—	5	21 ± 2	111 ± 12	56 ± 4
—	50	22 ± 1	58 ± 5	37 ± 3

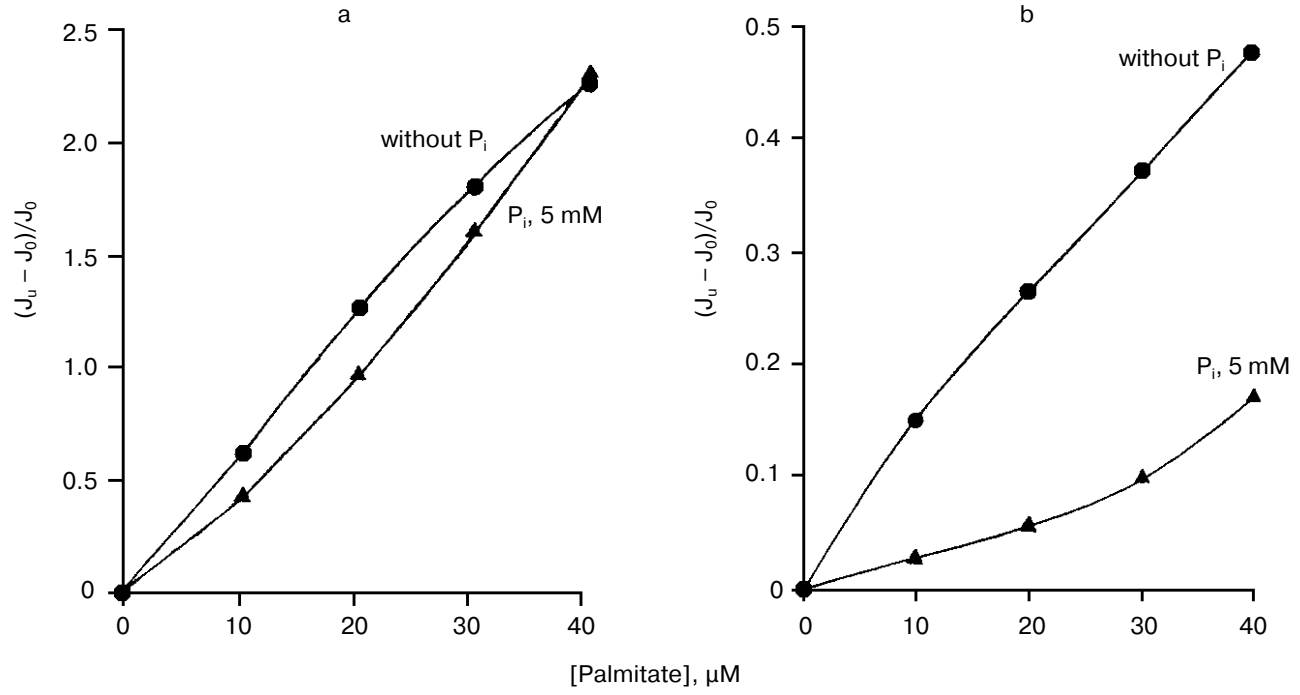

Fig. 4. Influences of phosphate on the uncoupling effect of palmitate at 25°C (a) and 13°C (b).

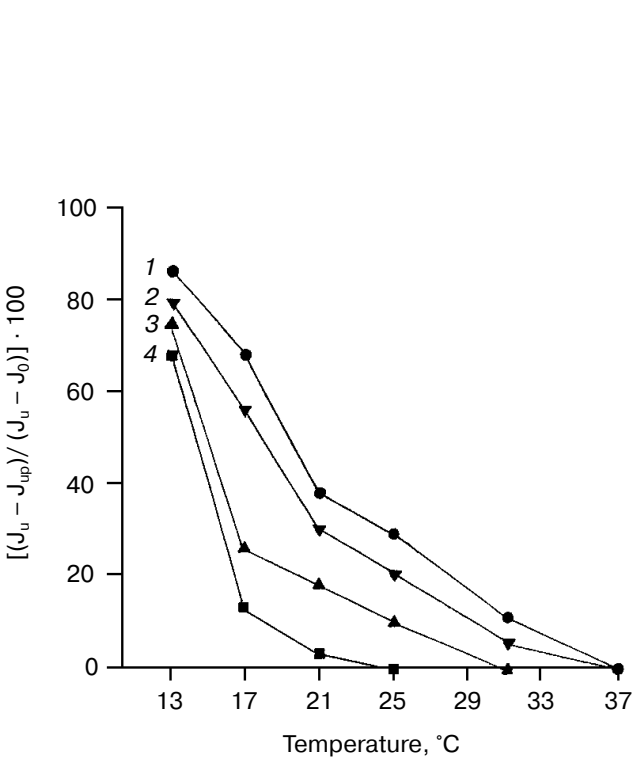

Fig. 5. Dependence of recoupling effects of phosphate (5 mM) on temperature on uncoupling with palmitate at the concentrations of 10 (*1*), 20 (*2*), 30 (*3*), and 40 μM (*4*).

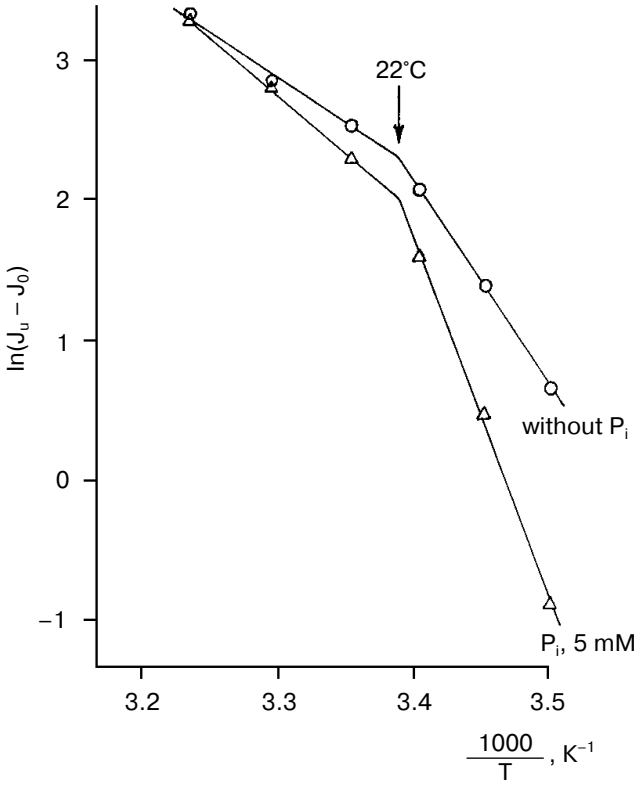

Fig. 6. Arrhenius plot of temperature dependences of the uncoupling effect of 20 μM palmitate in the presence and in the absence of phosphate (P_i).

Table 2. Effect of phosphate on the stimulation of mitochondrial respiration by 20 μM palmitate in the absence and in the presence of carboxyatractylate (Catr) and glutamate (Glu) at 19°C. Mean values ± error of the mean are presented ($n = 4$)

Additions	Stimulation of respiration, nmol O_2/min per mg protein	
	without phosphate	+ phosphate, 5 mM
—	12.1 ± 0.5	7.7 ± 0.4
Catr (1 μM)	6.6 ± 0.4	4.8 ± 0.3
Catr (1 μM) + Glu (2 mM)	2.2 ± 0.2	2.1 ± 0.2

by 36% the uncoupling effect of palmitate in the absence of carboxyatractylate and glutamate but had no influence in the concurrent presence of these two recoupling agents. In their turn, carboxyatractylate and glutamate in the presence of phosphate suppressed less the uncoupling effect of palmitate.

As mentioned above, the phosphate carrier can be involved in the uncoupling effect of fatty acids in yeast mitochondria. The recoupling effect of phosphate found by us suggested that the phosphate carrier should be involved in the uncoupling effect of fatty acids also in liver mitochondria. And in this case inhibitors of the phosphate carrier are also likely to have a recoupling effect. Mersalyl, in particular, is an inhibitor of the phosphate carrier and completely inhibits this carrier in liver mitochondria at the concentration of 10 nmol/mg protein [37, 38]. At a higher concentration (30 nmol/mg protein) mersalyl also inhibits the dicarboxylate carrier [39]. The inhibition of the phosphate carrier by mersalyl is accompanied by decrease in the rate of mitochondrial respiration in state 3 to the level of state 4 [37], whereas the inhibition of the dicarboxylate carrier suppressed the respiration in the course of succinate oxidation in any state of mitochondria [30]. We found earlier that mersalyl at the concentration of 10 nmol/mg protein suppressed the respiration in state 3 nearly to the state 4 level but very insignificantly inhibited the mitochondrial respiration in the course of succinate oxidation in the presence of 50 μM DNP (data not presented). Consequently, at this concentration mersalyl completely inhibited the phosphate carrier but slightly affected the dicarboxylate carrier. Mersalyl and phosphate similarly suppressed the laurate-induced stimulation of mitochondrial respiration, but their effects were not additive (Table 3). Unlike palmitate, the aspartate/glutamate antiporter was earlier shown to mainly contribute to the uncoupling effect of laurate [18]. Aspartate, a substrate of this carrier, effectively inhibited the uncoupling effect of fatty acids [15]. The recoupling effect of aspartate was markedly decreased under the influence of phosphate or mersalyl: in the absence of phosphate and mersalyl, aspartate inhibited by 47% the laurate-induced stimulation of respiration, in the presence of mersalyl the inhibition was 28%, and in the presence of phosphate the inhibition was 31% (Table 3). In their turns, phosphate and mersalyl significantly less suppressed the uncoupling effect of laurate in the presence of aspartate (Table 3).

DISCUSSION

The uncoupling effect of palmitate is shown to strongly depend on temperature, and this dependence has a distinct bend at 22°C in the Arrhenius plot (Fig. 2 and Table 1). It is unlikely that in the absence of fatty acids (in state 4) the rate of mitochondrial respiration depends on temperature significantly less, and in the Arrhenius plot this dependence is linear in the range from 13 to 37°C. In the latter case, the E_a value was only 17 kJ/mol, which is significantly less than the similar value for metabolite carriers and is specific for channels and free diffusion [40, 41]. As mentioned above, the res-

Table 3. Effect of mersalyl (10 nmol/mg protein) and phosphate (5 mM) on the stimulation of respiration of liver mitochondria by 30 μM laurate in the absence and in the presence of aspartate (Asp) at 19°C. Mean values ± error of the mean are presented ($n = 4$)

Additions	Stimulation of respiration, nmol O_2/min per mg protein			
	without phosphate and mersalyl	phosphate	mersalyl	phosphate + mersalyl
—	13.6 ± 0.8	9.0 ± 0.5	8.9 ± 0.3	9.1 ± 0.4
Asp, 0.8 mM	9.2 ± 0.7	7.8 ± 0.3	7.6 ± 0.2	8.0 ± 0.3
Asp, 3.2 mM	7.2 ± 0.7	6.2 ± 0.2	6.4 ± 0.2	6.3 ± 0.3

piration of mitochondria in state 4 was caused by reentry of protons into the matrix [30, 31]. Based on our findings, it was suggested that this process should be realized by diffusion of protons through some channels of the inner mitochondrial membrane.

Because anion carriers of the inner mitochondrial membrane are involved in the uncoupling effect of fatty acids [4, 5, 11-15], it was reasonable to expect that the temperature dependence of the uncoupling effect of fatty acids should resemble the temperature dependence of activity of the carriers. In fact, at temperatures higher than 22°C the E_a values of the carriers were very close to the E_a value observed for the uncoupling effect of palmitate (Table 1, [40-43]). However, unlike our findings, the temperature dependence of activity of the ADP/ATP-antiporter of liver mitochondria had a bend at 13.5°C [43] in the Arrhenius plot and the temperature dependence of activity of the aspartate/glutamate antiporter of liver mitochondria had no bend in the range from 1 to 35°C [42]. The bend in the Arrhenius plot near 20-22°C is specific for the temperature dependence of mobility of hydrophobic molecules incorporated in phospholipids of mitochondria, the spin-labeled and fluorescent probes, and, thus, for the temperature dependence of the mitochondrial phospholipid bilayer and also for the temperature dependence of mitochondrial cytochrome *c* reductase [44, 45]. The bend point is suggested to characterize a jump-like change in the fluidity of the mitochondrial membrane that can be due to the phase transition of lipids [44, 45]. The bend point at about 20°C in the Arrhenius plot was shown for the temperature dependence of mitochondrial respiration in state 3 and in the presence of various uncouplers [46]. However, in the case of maximal mitochondrial respiration, unlike the respiration in state 4 and with partial uncoupling, the bend point in the temperature curve may be determined by the change of the limiting stage of respiration [46].

Thus, the change in the fluidity of mitochondrial membrane induces functional changes in cytochrome *c* reductase [45], and, according to our data, correlates with changes in the uncoupling effect of palmitate but to a lesser degree causes functional changes in the substrate carriers [42, 43]. This seems to be due to different mechanisms of the catalytic action of the electron transport enzymes and substrate carriers. The electron transport is characterized by diffusion of a mobile carrier of coenzyme Q in the membrane [3]. Substrate carriers have specific features in structure and functions: six hydrophobic transmembrane α-helices surrounding hydrophilic structures involved in the transfer of hydrophilic substrates [47, 48]. Hydrophobic structures of the carriers are not very mobile, and only internal hydrophilic structures failing to interact with membrane lipids significantly change their conformations during the transport of a hydrophilic substrate [47, 48].

As has been said, the uncoupling was limited by the transport of fatty acid anions from the internal membrane monolayer onto the external one [4, 5, 11, 12, 21, 22]. Most likely, this transport occurred over the external surface of protein in the protein–lipid interphase, and a negatively charged carboxy group of fatty acid interacted with a low affinity site, whereas a hydrophobic acylic tail was located inside the phospholipid bilayer [12, 18]. With this in mind, it was suggested that the transport of fatty acid anions by carriers involved in the uncoupling, unlike the transport of their hydrophilic substrates, should significantly depend on the state of membrane lipids. This hypothesis is supported by our findings that the temperature dependence of stimulation of the respiration with partial uncoupling by DNP has the same characteristics as at the partial uncoupling by palmitate. Unlike the uncoupling effect of fatty acids, this effect of DNP significantly less depends on ADP/ATP- and aspartate/glutamate antiporters [15, 18]. The charge of the DNP anion is delocalized; therefore, unlike anions of fatty acids, DNP anions can move across the phospholipid bilayer without the involvement of proteins [49]. Therefore, the mobility of DNP anions will significantly depend on the membrane fluidity. In this case, equal E_a values seem to indicate that on translocation from the internal membrane monolayer onto the external one, anions of DNP and fatty acids overcome energy barriers of similar height.

In this work it is shown that at the temperature lower than the standard temperature of 25°C phosphate significantly suppressed the uncoupling effect of fatty acids. A nonspecific inhibitor of the phosphate carrier, mersalyl, suppressed the uncoupling effect of fatty acids similarly to phosphate and non-additively with it. These findings suggest the involvement of the phosphate carrier in the uncoupling effect of fatty acids in liver mitochondria. This hypothesis is consistent with the known data that fatty acids can interact with the phosphate carrier and inhibit the electroneutral transport of phosphate in liver mitochondria [20] and also with the report that the phosphate carrier from yeast mitochondria incorporated into liposomes can accelerate the transmembrane cyclic transport of protons by fatty acids [19, 20]. Note that phosphate can be transported across the mitochondrial membrane also by the dicarboxylate carrier [39, 40], and mersalyl at the higher concentration than used by us inhibits this carrier [39]. However, the involvement of the dicarboxylate carrier in the uncoupling in the presence of high concentration of succinate is unlikely, because this carrier is known to be involved in the uncoupling effect of fatty acids only in the absence of succinate [16].

At first glance, the hypothesis on the involvement of the phosphate carrier in the uncoupling effect of fatty acids contradicts the observation of the recoupling effects of phosphate and mersalyl only in the absence of carboxyatractylate and glutamate (or aspartate). In fact,

if with uncoupling by fatty acids the phosphate carrier is considered only as an additional pathway of the proton reentry into the matrix independently of two other pathways which involve the ADP/ATP- and aspartate/glutamate antiporters, the effects of phosphate and mersalyl are to be additive with respect to the effects of carboxyatractylate and glutamate (or aspartate). The findings would be considered as supporting the influence of phosphate and mersalyl on the ADP/ATP- and aspartate/glutamate antiporters. However, this contradicts the inability of phosphate to interact with these carriers. Mersalyl interacts with SH-groups of the ADP/ATP-antiporter [50] and the aspartate/glutamate antiporter [41], but at concentration an order higher than the concentration used by us. Moreover, in experiments with the ADP/ATP-antiporter incorporated into proteoliposomes the interaction of mersalyl with the SH-group of this carrier is shown not to weaken but, on the contrary, to increase the protonophore activity of fatty acids [50].

This contradiction can be escaped on the assumption that the phosphate carrier is involved in the uncoupling only in a complex with either the ADP/ATP-antiporter or the aspartate/glutamate antiporter. The phosphate carrier plays in these uncoupling complexes only a subsidiary role. This carrier located near one of these antiporters ensures additional sites for fatty acid anions in a hydrophobic region of the membrane. The displacement of these anions from the external to the internal layer of the membrane is similar to passing on the baton from the sites of one carrier to the sites of the other. This decreases the energy barrier for fatty acid anions at their transmembrane transfer. Phosphate or mersalyl anions eliminate these additional binding sites by neutralization of positive charges of the carrier. As a result, the height of the energy barrier is increased again.

This work was supported by the Russian Foundation for Basic Research (projects No. 01-04-48317 and No. 02-04-06333) and by the Interuniversity Scientific Program "University of Russia" (project No. 07.01.049).

REFERENCES

1. Brand, M. D., Harper, M.-E., and Taylor, H. C. (1993) *Biochem. J.*, **291**, 739-748.
2. Rolfe, D. F. S., and Brand, M. D. (1997) *Biosci. Rep.*, **17**, 9-16.
3. Skulachev, V. P. (1989) *Energetics of Biological Membranes* [in Russian], Nauka, Moscow.
4. Skulachev, V. P. (1998) *Biochim. Biophys. Acta*, **1363**, 100-124.
5. Skulachev, V. P. (1999) *Mol. Asp. Med.*, **20**, 139-184.
6. Boss, O., Muzzin, P., and Giacobino, J. P. (1998) *Eur. J. Endocrinol.*, **139**, 1-9.
7. Belyaeva, E. A., and Wojtczak, L. (1994) *Biochem. Mol. Biol. Int.*, **33**, 165-175.
8. Chavin, K. D., Yang, S. Q., Lin, H. Z., Chatham, J., Chacho, V. P., Hoek, J. B., Walajtys-Rode, E., Rashid, A., Chen, Ch.-H., Huang, Ch.-Ch., Wu, T. Ch., Lane, M. D., and Diehl, A. M. (1999) *J. Biol. Chem.*, **274**, 5692-5700.
9. Bilenko, M. V. (1989) *Ischemia and Reperfusion-Induced Organ Damages* [in Russian], Meditsina, Moscow.
10. Lenton, L. M., Behm, C. A., and Bygrave, F. L. (1995) *Biochem. J.*, **307**, 425-431.
11. Skulachev, V. P. (1999) *J. Bioenerg. Biomembr.*, **31**, 431-445.
12. Wojtczak, L., and Wieckowski, M. R. (1999) *J. Bioenerg. Biomembr.*, **31**, 447-455.
13. Samartsev, V. N. (2000) *Biochemistry (Moscow)*, **65**, 991-1005.
14. Andreyev, A. Yu., Bondareva, T. O., Dedukhova, V. I., Mokhova, E. N., Skulachev, V. P., Tsofina, L. M., Volkov, N. I., and Vygodina, T. V. (1989) *Eur. J. Biochem.*, **182**, 585-592.
15. Samartsev, V. N., Smirnov, A. V., Zeldi, I. P., Markova, O. V., Mokhova, E. N., and Skulachev, V. P. (1997) *Biochim. Biophys. Acta*, **1339**, 251-257.
16. Wieckowski, M. R., and Wojtczak, L. (1997) *Biochem. Biophys. Res. Commun.*, **232**, 414-417.
17. Samartsev, V. N., Mokhova, E. N., and Skulachev, V. P. (1997) *FEBS Lett.*, **412**, 179-182.
18. Samartsev, V. N., Markova, O. V., Zeldi, I. P., and Smirnov, A. V. (1999) *Biochemistry (Moscow)*, **64**, 901-911.
19. Zackova, M., Kramer, R., and Jezek, P. (2000) *Int. J. Biochem. Cell. Biol.*, **32**, 499-508.
20. Engstova, H., Zackova, M., Ruzicka, M., Meinhardt, A., Hanus, J., Kramer, R., and Jezek, P. (2001) *J. Biol. Chem.*, **276**, 4683-4691.
21. Skulachev, V. P. (1991) *FEBS Lett.*, **294**, 158-162.
22. Jezek, P., Engstova, H., Zackova, M., Vercesi, A. E., Costa, A. D. T., Arruda, P., and Garlid, K. D. (1998) *Biochim. Biophys. Acta*, **1365**, 319-327.
23. Schonfeld, P. (1992) *FEBS Lett.*, **303**, 190-192.
24. Markova, O. V., Bondarenko, D. I., and Samartsev, V. N. (1999) *Biochemistry (Moscow)*, **64**, 565-570.
25. Keleti, T. (1990) *Basic Enzyme Kinetics* [Russian translation], Mir, Moscow.
26. Wieckowski, M. R., and Wojtczak, L. (1998) *FEBS Lett.*, **423**, 339-342.
27. Chavez, E., Zazueta, C., and Garcia, N. (1999) *FEBS Lett.*, **445**, 189-191.
28. Sharpe, M. A., Cooper, C. E., and Wrigglesworth, J. M. (1994) *J. Membr. Biol.*, **141**, 21-28.
29. Wojtczak, L., and Schonfeld, P. (1993) *Biochim. Biophys. Acta*, **1183**, 41-57.
30. Groen, A. K., Wanders, R. J. A., Westerhoff, H. V., van der Meer, R., and Tager, J. M. (1982) *J. Biol. Chem.*, **257**, 2754-2757.
31. Samartsev, V. N., and Polishchuk, L. S. (2002) *Biol. Membr. (Moscow)*, **19**, 232-237.
32. Westerhoff, H., and van Dam, K. (1992) *Thermodynamics and Control of Biological Free-Energy Transduction* [Russian translation], Mir, Moscow.
33. Zoratti, M., and Petronilli, V. (1985) *FEBS Lett.*, **193**, 276-282.
34. Woelders, H., Putters, J., and van Dam, K. (1986) *FEBS Lett.*, **204**, 17-21.
35. Schonfeld, P., Schild, L., and Kunz, W. (1989) *Biochim. Biophys. Acta*, **977**, 266-272.

36. Shinohara, Ya., Unami, A., Teshima, M., Nishida, H., van Dam, K., and Terada, H. (1995) *Biochim. Biophys. Acta*, **1228**, 229-234.
37. Fonyo, A. (1979) *Pharmacol. Ther.*, **7**, 627-645.
38. Ligeti, E., Brandolin, G., Dupont, Y., and Vignais, P. V. (1985) *Biochemistry*, **24**, 4423-4428.
39. LaNoue, K. F., and Schoolwerth, A. C. (1979) *Ann. Rev. Biochem.*, **48**, 871-922.
40. Shol'ts, K. F. (1994) *Usp. Biol. Khim.*, **34**, 167-187.
41. Herick, K., and Kramer, R. (1995) *Biochim. Biophys. Acta*, **1238**, 63-71.
42. Tischler, M. E., Pachence, J., Williamson, J. R., and LaNoue, K. F. (1976) *Arch. Biochem. Biophys.*, **173**, 448-462.
43. Klingenberg, M., Grebe, K., and Appel, M. (1982) *Eur. J. Biochem.*, **126**, 263-269.
44. Rottenberg, H. (1978) *FEBS Lett.*, **94**, 295-297.
45. Augee, M. L., Pehowich, D. J., Raison, J. K., and Wang, L. C. H. (1984) *Biochim. Biophys. Acta*, **776**, 27-36.
46. Shol'ts, K. F., Aliverdieva, D. A., and Kotel'nikova, A. V. (1983) *Dokl. Akad. Nauk SSSR*, **273**, 747-750.
47. Walker, J. E., and Runswick, M. J. (1993) *J. Bioenerg. Biomembr.*, **25**, 435-446.
48. Brandolin, G., Le Saux, A., Trezeguet, V., Lauquin, G. J. M., and Vignais, P. V. (1993) *J. Bioenerg. Biomembr.*, **25**, 459-472.
49. Terada, H. (1981) *Biochim. Biophys. Acta*, **639**, 225-242.
50. Brustovetsky, N., and Klingenberg, M. (1994) *J. Biol. Chem.*, **269**, 27329-27336.