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Formation of Palmitic Acid/Ca^{2+} Complexes in the Mitochondrial Membrane: A Possible Role in the Cyclosporin-Insensitive Permeability Transition

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A possible role of palmitic acid/Ca^{2+} (PA/Ca^{2+}) complexes in the cyclosporin-insensitive permeability transition in mitochondria has been studied. It has been shown that in the presence of Ca^{2+}, PA induces a swelling of mitochondria, which is not inhibited by cyclosporin A. The swelling is accompanied by a drop in membrane potential, which cannot be explained only by a work of the Ca^{2+} uniporter. With time, the potential is restored. Evidence has been obtained indicating that the specific content of mitochondrial lipids would favor the PA/Ca^{2+}-induced permeabilization of the membrane. In experiments with liposomes, the PA/Ca^{2+}-induced membrane permeabilization was larger for liposomes formed from the mitochondrial lipids, as compared to the azolectin liposomes. Additionally, it has been found that in mitochondria of the TNF (tumor necrosis factor)-sensitive cells (WEHI-164 line), the content of PA is larger than in mitochondria of the TNF-insensitive cells (C6 line), with this difference being mainly provided by PA incorporated in phosphatidylethanolamine and especially, cardiolipin. The PA/Ca^{2+}-dependent mechanism of permeability transition in mitochondria might be related to some pathologies, e.g. myocardial ischemia. The heaviness of myocardial infarction of ischemic patients has been demonstrated to correlate directly with the content of PA in the human blood serum.

KEY WORDS: Mitochondria; palmitic acid; calcium binding; liposomes; CsA-insensitive pore; TNF.

INTRODUCTION

The role of palmitic (PA) and stearic (SA) acids as natural factors promoting apoptosis and necrosis of cells is widely discussed now (De Pablo *et al*., 1999; Kong and Rabkin, 2000; Sparagna *et al*., 2000). It has been established that these fatty acids can activate apoptosis by both the caspase-dependent and caspase-independent mechanisms (Ulloth *et al*., 2003). Such destructive processes as myocardial infarction have been shown to be accompanied by an increase in the concentration of fatty acids in serum (Oliver *et al*., 1968).

One of the ways of the apoptosis induction is the increase in permeability of the mitochondrial membrane, resulting in the release of proapoptotic proteins (Bernardi, 1999). Entering cytoplasm, these proteins activate enzymes, which trigger the process of programmed cell death. The mechanism by which PA and SA alter the permeability of mitochondrial membrane is under study now. There is a supposition that the membranotropic effect of

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Key to abbreviation: BLM, black-lipid membranes; CsA, cyclosporin A; FFA, free fatty acids; MPT, mitochondrial permeability transition; PA, palmitic acid; SA, stearic acid; TNF, tumor necrosis factor; SRB, sulforhodamine B; TPP^+, tetraphenylphosphonium; $\Delta\psi$, mitochondrial potential.

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these fatty acids is related to their ability to act as uncouplers (Skulachev, 1991), as is known that the fall of potential on the mitochondrial membrane ($\Delta\Psi$) favors, in the presence of Ca^{2+}, the opening of the cyclosporin A (CsA)-sensitive pore (Bernardi, 1999).

At the same time, we showed that PA and SA bound Ca^{2+} with high affinity ($K_d = 5 \times 10^{-6}$ M), while other fatty acids and lipids were ineffective (Mironova *et al.*, 2001). Binding Ca^{2+} in the lipid bilayer, these fatty acids enhance the permeability of BLM and liposomes for ions and substances with low molecular weight (Agafonov *et al.*, 2003; Mironova *et al.*, 2001). The addition of PA and SA to cells has been found to result in the release of cytochrome *c* and the induction of apoptosis (De Pablo *et al.*, 1999). It has been also established that the treatment of mitochondria with PA and Ca^{2+} leads to the swelling of these organelles (Sultan and Sokolove, 2001a,b).

This work continues study of the pore-forming effect of PA/Ca^{2+} complexes on the membranes of mitochondria and liposomes. The data on the PA/Ca^{2+}-induced membrane permeabilization are related to the examining of the composition and content of free fatty acids in mitochondria. The data obtained allow one to suppose that the increase of PA content in the mitochondrial membrane, leading to its permeabilization, can be a physiologically relevant mechanism and might be involved in development of such pathologies as myocardial ischemia.

MATERIALS AND METHODS

Experiments were conducted on mitochondria isolated from the liver of white rats (animal's weight was 180–220 g) using a convenient technique of differential centrifugation (Gateau-Roesch *et al.*, 2000). The isolation medium contained 210 mM mannitol, 70 mM sucrose, and 10 mM Hepes/KOH buffer (pH 7.4).

The swelling of mitochondria was followed by the absorbance change at 540 nm in a stirred and thermostated cuvette (25°C) using a "Uvikon" spectrophotometer. The incubation medium contained 210 mM mannitol, 70 mM sucrose, 5 mM succinic acid, 50 μM EGTA, and 10 mM Hepes/KOH buffer (pH 7.4). Also, 0.8 μM rotenone was added in the cuvette just before measurements.

$\Delta\Psi$ was estimated by following the TPP^+ distribution across the mitochondrial membrane; in this case, the incubation medium was supplemented with 1 μM TPP^+. The concentration of TPP^+ was measured with a TPP^+-sensitive electrode (Kamo *et al.*, 1979).

Lipids were extracted from mitochondria by the modified technique of Bligh and Dyer (1959), which is a simplified variant of the classical Folch technique. One milliliter of diluted mitochondrial suspension (50 mg of protein/mL) was placed in a centrifuge tube and 3.75 mL of chloroform/methanol (1:2) mixture were added. After incubation at 4°C for 1–2 h (with periodical shaking), the mixture was centrifuged at 5500*g* for 15 min. The pellet was resuspended in 4.75 mL of chloroform/mehtanol/H_2O mixture, and the procedures of incubation at 4°C and centrifugation were repeated. The supernatants obtained from the two centrifugations were combined, added with 2.5 mL of chloroform and 2.5 mL of H_2O, and centrifuged to separate the chloroform layer from the water–methanolic one. After separation, the chloroform extract was added with an equal volume of benzol and dried under nitrogen at 30°C. Lipids were then dissolved in a known volume of chloroform/methanol (1:1) mixture and stored under nitrogen at −20°C.

Preparation of liposomes (large unilamellar vesicles) loaded with sulforhodamine B (SRB) and measurement of their permeabilization were made as described earlier (Agafonov *et al.*, 2003).

The tumor necrosis factor (TNF)-sensitive murine fibrosarcoma cell line WEHI-164 from ATCC (Rockeville, MD, USA) and the TNF-resistant C6 glioma cell line from E.C.A.C.C. (Salisbury, UK) were respectively cultured in commercial RPMI-1640 and DMEM media (with L-glutamine) supplemented with 10% foetal calf serum and containing penicillin (100 U/mL), streptomycin (100 μg/mL), and nonessential amino acids (only used for WEHI-164 cells). The cell lines were routinely tested for mycoplasma.

Isolation of mitochondria from WEHI-164 and C6 cells was performed as described earlier (Levrat and Louisot, 1996). Proteins were measured by the bincichonic acid assay (Smith *et al.*, 1985), using bovine serum albumin as a standard.

Prior lipid extraction, confluent cells (3×108) were washed twice with HBSS, then harvested and washed three times with PBS. Cells or 500 μg of mitochondria were subjected to a Folch extraction (Folch *et al.*, 1957).

Free fatty acids were isolated using aminopropyl-bonded silica columns and eluted with a mixture of diethylether/acetic acid and analyzed by gas chromatography after methylation (Pietsch and Lorenz, 1993).

Mitochondrial phospholipid were analyzed by two-dimensional thin layer chromatography (Rouser *et al.*, 1970). Total lipid extracts from mitochondria were applied on silica gel 60 plates (10×20 cm), then they were developed twice in the first direction with chloroform/methanol/28% ammonia (65:25:4, v/v) and in the second direction with chloroform/acetone/methanol/acetic acid/water (30:40:10:10:5, v/v). Developed chromatograms were stained with iodide or 0.02%

2′,7′-dichlorofluoroscein in ethanol/water (95:5, v/v). The areas corresponding to the isolated phospholipids were scrapped off and the phospholipid amounts were estimated by the inorganic phosphorus determination (Bartlett, 1959). The fatty acid content of each phospholipid was determined by gas chromatography as described previously (Levrat and Louisot, 1996).

RESULTS

Effect of Lipid Composition, Ionic Strength, and Osmolarity on the PA/Ca^{2+}-Induced Permeabilization of Artificial Membranes

As was established earlier, 30–40 μM PA caused the 30% Ca^{2+}-induced release of SRB from azolectin liposomes (Agafonov *et al.*, 2003). When the mitochondrial lipids were used to form liposomes, the SRB release increased up to 70% under the same conditions (Fig. 1(A) and (B)). With Ca^{2+} concentration raised to 1 mM, an almost complete release of SRB from liposomes was observed. As in case of azolectin liposomes, the release of SRB from liposomes, made of mitochondrial lipids, developed only when Ca^{2+} was added before PA. The dependence of PA/Ca^{2+}-induced permeabilization of liposomes, formed from mitochondrial lipids, on the concentration of PA and Ca^{2+} is presented in Fig. 1(B) and (C). The character of these curves indicates that it is the formation of PA/Ca^{2+} complexes which causes the membrane permeabilization. The maximal SRB release was observed at the concentrations of PA and Ca^{2+}, equal to 40 μM and 0.4 mM, respectively; the half maximal—at the concentrations of 20 and 50 μM correspondingly.

It was found that the raise in ionic strength (up to 150 mM KCl) and osmolarity (up to 300 mM sucrose) of the medium had a little effect on the PA/Ca^{2+}-induced release of SRB from liposomes (Fig. 2).

PA/Ca^{2+}-Induced Swelling of Mitochondria

The experiments on intact mitochondria, examining the effect of PA and Ca^{2+}, were performed taking into account data on the half maximal PA/Ca^{2+}-induced permeabilization of liposomes. The addition of 15 μM PA in the presence of 50 μM EGTA did not lead to the swelling of mitochondria (Fig. 3). But when 30 μM Ca^{2+} was added next, a swelling was observed, which was not inhibited by CsA.

During swelling of mitochonsdria a decrease in $\Delta\Psi$ was observed (Fig. 4). This decrease had a temporal character: $\Delta\Psi$ was restoring with time. It should be noted that in the absence of Ca^{2+}, PA also caused a slight $\Delta\Psi$ drop, but no swelling of mitochondria occurred. This effect of PA was possibly related to its ability to act as an uncoupler (Skulachev, 1991). With time, however, this drop

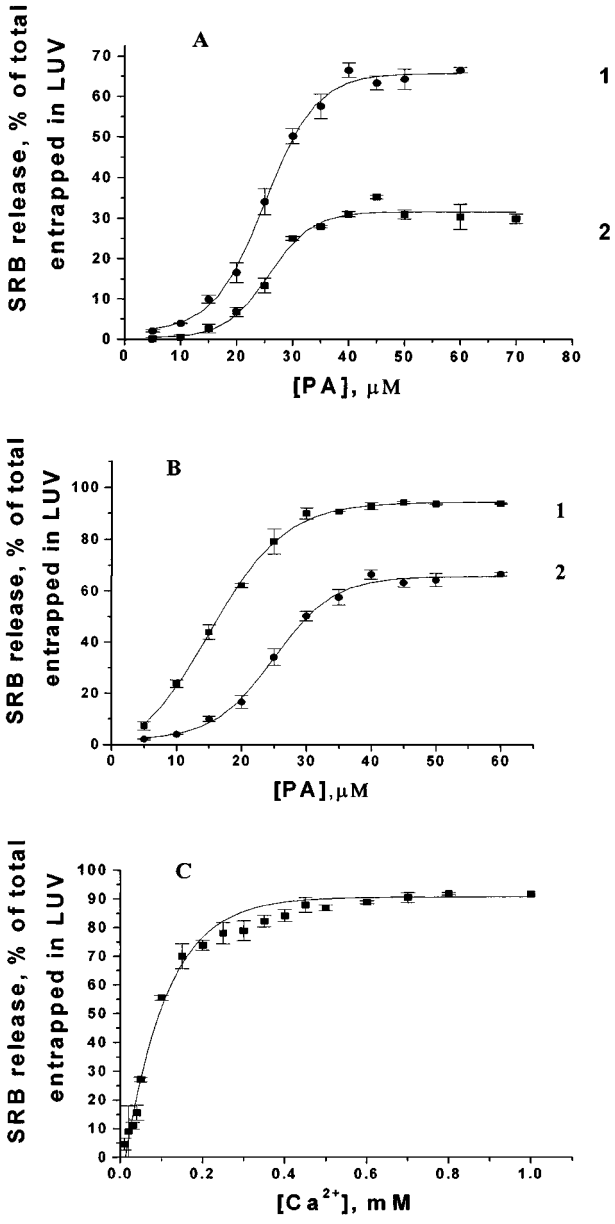

Fig. 1. Dependence of PA/Ca^{2+}-induced SRB release from liposomes on the concentration of PA (A and B) and Ca^{2+} (C). The medium contained 10 mM Tris-HCl buffer (pH 8.5), 50 μM EGTA, and 40 mM KCl. Liposomes were formed from either mitochondrial lipids (A-1, B, and C) or from azolectin (A-2). The concentration of lipid in all cases was 10 mg/ml. a - The concentration of Ca^{2+} was 0.1 mM; B - the concentration of Ca^{2+} was 1 mM (B-1) or 0.1 mM (B-2); (B) the concentration of Ca^{2+} was 0.1 mM; (C) the concentration of PA was 50 μM.

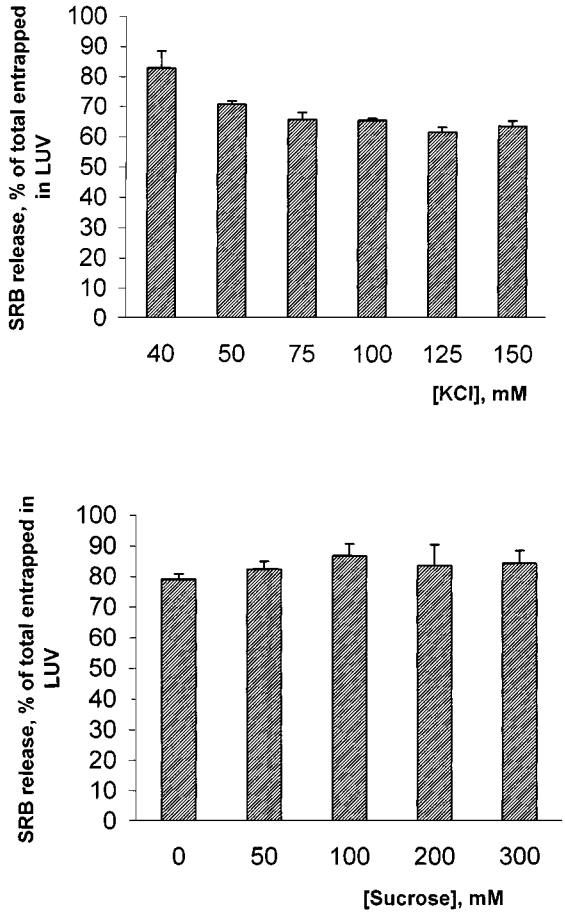

Fig. 2. The effect of osmolarity and ionic strength of the medium on the PA/Ca^{2+}-induced SRB release from azolectin liposomes. The medium composition was as in Fig. 1. The concentration of PA was 50 μM and Ca^{2+} 1 mM.

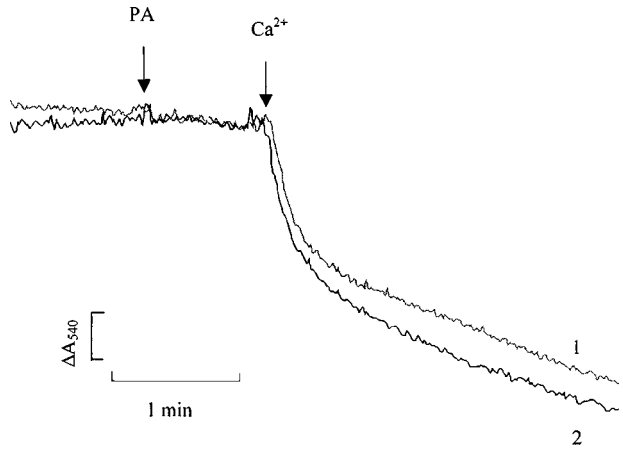

Fig. 3. PA/Ca^{2+}-induced swelling of rat heart mitochondria in the absence (1) or presence (2) of 1 μM CsA. The incubation medium contained 5 mM succinate, 0.8 μM rotenone, 210 mM mannitol, 70 mM sucrose, 50 μM EGTA, and 10 mM Hepes-KOH (pH 7.4). The concentration of mitochondrial protein was 0.4 mg/mL. Additions: 15 μM PA (9.6 μg/mg of protein), 80 μM Ca^{2+} (the concentration of free Ca^{2+} was about 30 μM), and $\Delta A_{540} = 0.1$.

was removing too (Fig. 4(B)). The restoration of $\Delta\Psi$ occurred differently in the cases of PA/Ca^{2+}-induced drop and Ca^{2+}-induced drop. Hence, the PA/Ca^{2+}-induced $\Delta\Psi$ drop cannot be explained only by a Ca^{2+}-uniporter work. When the swelling of mitochondria came to the end, a gradual closing of the pore was observed—eventually, $\Delta\Psi$ seemed to be completely restored (Fig. 4(B)).

Possible Lipid Sources for PA in Mitochondria

The increase in mitochondrial PA content can be observed in vivo in the case of activation of phospholipases. One of the factors that activate mitochondrial phospholipase A_2 is the tumor necrosis factor, TNF (Levrat and Louisot, 1996). There are cells, which are sensitive to TNF (WEHI-164), and cells, which are TNF-insensitive (C6). The comparison of free fatty acid (FFA) composition in these cell lines shows that the content of free PA in the mitochondria of C6 cells is 2.5 times lower than that in the mitochondria of WEHI-164 cells. As can be seen from Table I, this difference is mainly related to the mitochondria: in the whole cell, it is less pronounced. The difference in SA content, although being smaller, has the same character.

Examining the content of PA incorporated in phospholipids of both cell lines demonstrates that the quantity of PA in phosphatydilethanolamine and especially, cardiolipin is substantially higher in the case of TNF-sensitive cells, comparing with the TNF-insensitive ones (Table II). Differences in the SA content have the same character. At the same time, WEHI-164 and C6 cells almost do not differ in the content of phosphatidylcholine-incorporated PA and SA. Probably, the high PA and SA content in cardiolipin and phosphatydilethanolamine of WEHI-164 cells is related to the high content of free PA and SA in these cells.

Content of Various Free Fatty Acids in the Mitochondria and Blood Serum and Alterations of the Free Fatty Acid Content in Serum Upon the Myocardial Infarction

In heart mitochondria, the content of saturated fatty acids is somewhat larger than that of unsaturated ones (Table III). Small differences in the content of fatty acids,

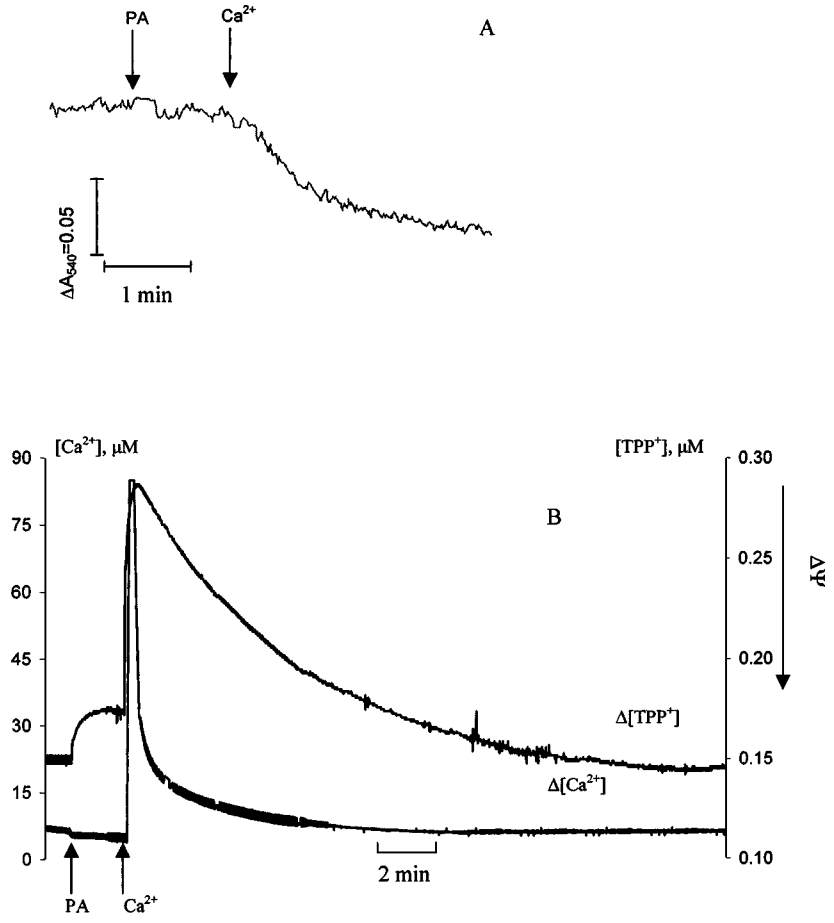

Fig. 4. Swelling of mitochondria (A) and changes of $\Delta\Psi$ and Ca^{2+} concentration (B) in the response on additions of palmitic acid (PA) and Ca^{2+}. The concentration of mitochondrial protein to 2 mg/mL. The additions: 30 μM PA, 120 μM Ca^{2+} (the concentration of free Ca^{2+} was about 70 μM). The medium of incubation was the same as in Fig. 3 and supplemented with 1 μM CsA.

observed for different animals, are probably related with the diet and the type of tissue. Nevertheless, the principal regularities, described below, hold true for all control animals (Table III; Kotani *et al*., 2000). Among saturated fatty acids in mitochondria, the main species are PA (C16:0), SA (C18:0), docosanoic (C22:0), and lignoceric (C24:0) acids. According to our data (Mironova *et al*., 2001), all these fatty acids bind Ca^{2+} quite well. Lauric and myristic acids, whose ability to bind Ca^{2+} is significantly lower, are almost absent in mitochondria. As for unsaturated fatty acids, these are only oleic and linoleic acids, whose quantities in mitochondria are sufficient; and according to our data, these fatty acids almost do not bind Ca^{2+}. Other unsaturated fatty acids are presented in mitochondria in trace amounts.

Table I. The Content of Free Palmitic and Stearic Acids in C6 and WEHI-164 Cells

	Content (μg/mg)			
	Palmitic acid		Stearic acid	
	C6	WEHI-164	C6	WEHI-164
Total lipid extract	8.6 ± 0.4	12.2 ± 0.0	6.5 ± 0.6	6.4 ± 0.0
Mitochondrial lipid extact	7.1 ± 0.3	17.5 ± 0.8	5.8 ± 1.3	11.0 ± 0.1

Note. The values given are averages of five samples with standard deviations.

It is known that the content of FFA in the cell membranes are related to their content in the blood. It was shown that after addition of radiolabeled PA to the culture medium, 1 h was needed for PA to incorporate into the mitochondrial phospholipids (Jakobsson *et al*., 1990). The increase of PA or SA concentration in the blood above the control level can be dangerous for life: a similar increase of PA concentration in the cell cultivation medium

Table II. The Content of Palmitic and Stearic Acids in Some Lipids of C6 and WEHI-164 Cells[a]

Lipid	Palmitic acid (μg/μg Pi)		Stearic acid (μg/μg Pi)	
	C6	WEHI-164	C6	WEHI-164
Phosphatidylcholine	6.1	5.8	1.5	1.3
Phosphatidylethanolamine	1.1	1.7	3.5	6.5
Sphingomyelin	4.3	5.4	1.4	0.7
Cardiolipin	0.51 ± 0.07	4.08 ± 0.28	0.49 ± 0.18	1.08 ± 0.18

[a] Statistical processing was done for cardiolipin only; the values given for cardiolipin are averages of five samples with standard deviations.

was shown to lead to the necrosis of cardiomyocytes (Kong and Rabkin, 2000).

The main fatty acids, presented in the blood of a healthy man, are PA, SA, oleic, and docosanoic acids (Table III). In the blood of ischemic patients, the content of all these fatty acids (excluding docosanoic one) increases significantly (Table IV). This increase in the fatty acid content may be either a cause or a consequence of myocardial infarction. As known, the main indicator for the development of myocardial infarction is the content of troponin I in the blood. Our assays show that there is a direct correlation between the contents of troponin I and some fatty acids (PA, SA, and oleic acid) in the serum of ischemic patients (Table IV). Changes in the concentration of PA and oleic acid are higher than changes in the concentration of SA. Variations in the concentration of docosanoic acid are irregular.

Table III. The Content of Free Fatty Acid Species in the Mitochondria and Blood Serum[a]

Free fatty acids	Rat liver mitochondria		Human serum	
	μg/mg of protein	% mol	μg/mL	% mol
14:0	0.89	1.74	0.0	0.0
16:0	10.14	22.21	64.7	32.0
16:1 n-9	0.17	0.40	0.0	0.0
16:1 n-7	0.11	0.27	0.0	0.0
18:0	10.98	26.16	23.7	11.1
18:1 n-9	8.47	20.52	60.4	28.8
18:1 n-7	0.00	0.00	0.0	0.0
18:2 n-6	3.79	9.16	26.0	12.2
18:3 n-3	0.21	0.53	0.0	0.0
20:0	0.40	1.14	0.0	0.0
20:3 n-6	0.10	0.27	0.0	0.0
22:0	3.02	8.80	30.9	15.9
20:4 n-6	0.10	0.27	0.0	0.0
20:5 n-3	0.28	0.80	0.0	0.0
24:0	0.14	0.50	0.0	0.0
22:4 n-6	2.21	5.81	0.0	0.0
22:5 n-6	0.14	0.42	0.0	0.0
22:5 n-3	0.12	0.37	0.0	0.0
22:6 n-3	0.21	0.63	0.0	0.0

[a] The values given are averages of 6–8 samples; their standard deviations were about 5%.

DISCUSSION

The specific ability of PA and SA to bind Ca^{2+} with high affinity, which has been found recently (Mironova *et al*., 2001), gives a new insight in the mechanism of fatty acid-induced changes in the permeability of mitochondrial membrane. Earlier, these fatty acids were mainly considered as uncouplers of oxidative phosphorylation (Skulachev, 1991). However, the data on the Ca^{2+}-dependent effect of PA and SA on the permeability of artificial membranes (Agafonov *et al*., 2003; Mironova *et al*., 2001) suggest that the mechanism of PA- or SA-induced permeability changes in the mitochondrial membrane should be related to the formation of PA/Ca^{2+} or SA/Ca^{2+} complexes in the lipid bilayer.

Apparently, the PA/Ca^{2+}-induced energy-dependent swelling of mitochondria, which may lead to the release of cytochrome *c* and the induction of apoptosis (Kong and Rabkin, 2000), does result from the formation of PA/Ca^{2+} complexes in the mitochondrial membrane (Figs. 3 and 4; Agafonov *et al*., 2003; Mironova *et al*., 2001; Sultan and

Table IV. The Content of Major Free Fatty Acid Species in the Human Blood Serum in Relation to the Concentration of Troponin I

Sample no.[a]	Troponine I (μg/l)	Free fatty acid (μg/mL)			
		C16:0	C18:0	C18:1 n-9	C22:0
1	0.05	58.4	39.5	38.6	96.1
2	8.84	92.8	52.7	111.2	0.0
3	17.73	103.6	63.7	130.6	0.0
4	18.57	103.8	68.1	159.5	0.0
5	153.10	139.6	42.2	186.1	49.2
6	203.70	153.7	46.7	166.9	48.0
7	323.10	217.1	118.4	227.9	38.4
8	350.40	237.4	90.0	284.9	6.0

[a] 1: a healthy man; 2–8: patients on different stages of ischemia.

Sokolove, 2001a,b). When mitochondria swell in the presence of PA and Ca^{2+}, $\Delta\Psi$ decreases, but with time (within 10 min), it is restored up to the initial level (Fig. 4). These changes of $\Delta\Psi$ seem to have no connection with the work of Ca^{2+}-uniporter, because under these conditions, Ca^{2+} is accumulated in mitochondria within a minute, while the restoration of $\Delta\Psi$ occurs rather slowly (Fig. 4). The gradual restoration of $\Delta\Psi$ will begin when the swelling of mitochondria has almost completed. The restoration of $\Delta\Psi$ indicates that the changes in the permeability of mitochondrial membrane are reversible. However, the volume of mitochondria is not restored within that time interval. It should be noted that under conditions of the experiment presented in Fig. 4, the decrease of $\Delta\Psi$ does not exceed 30%. The fact that the opening of PA/Ca^{2+}-induced pore is temporal was also demonstrated in the laboratory of Sokolove using polyethyleneglycols of different molecular weights (Sultan and Sokolove, 2001a,b).

The fact that the drop in $\Delta\Psi$ is restored with time testifies to a possible functional significance of the CsA-insensitive PA/Ca^{2+}-dependent MPT. It is known that in the final stage of apoptosis, mitochondria usually keep some functionality, the latter is important for the cell membrane to be intact (Kerr *et al*., 1972; Majno and Joris, 1995). However, if mitochondria degrade irreversibly, this would lead to the necrotic cell death (Crompton, 1999). Therefore, the PA/Ca^{2+}-induced permeabilization of the mitochondrial membrane, which does not lead to the degradation of mitochondria but, probably, results in the release of cytochrome *c* and other proapoptotic proteins, should be a functionally relevant trigger of apoptosis. However, a too high content of free long-chain saturated fatty acids in mitochondria may cause a persistent collapse of $\Delta\Psi$, which can be followed by the degradation of organelles (Bernardi, 1999) and the subsequent death of a cell by the necrotic pathway.

The functional relevance of CsA-insensitive PA/Ca^{2+}-dependent MPT is also supported by the fact that the mitochondrial membrane appears to be sort of predisposed to the development of this phenomenon. First, the composition of mitochondrial lipids seems to be more preferable for the PA/Ca^{2+}-induced permeabilization of lipid bilayer. When vesicles were formed from the mitochondrial lipids instead of azolectin, the PA/Ca^{2+}-induced release of SRB from liposomes turned out to be quite higher (Fig. 1). Similar results concerning comparison between the mitochondrial and total brain lipids were obtained earlier in the experiments on BLM (Mironova *et al*., 2001).

Second, among FFA of the mitochondrial membrane, the saturated ones amount to the major part, with PA and SA being the predominant species (Table III). The content of these FFA in mitochondria seems to be already not very far from a threshold of the CsA-insensitive MPT pore opening, because the quantity of exogenous PA needed to open this pore is of the same order of magnitude as the endogenous PA level, about 10 μg/mg of protein (Fig. 3, Table III). Hence, the activation of phospholipases, usually taking place at pathological states, can add that last straw in the FFA pool of the mitochondrial membrane, which will trigger the CsA-insensitive MPT. In this respect, of interest are the measurements performed on the cells differing in their sensitivity to TNF, which is known to activate phospholipase A_2 (Levrat and Louisot, 1996). Our data show that the content of free PA and SA in the mitochondria of WEHI-164 cells is substantially higher than that in the mitochondria of C6 cells (Table I). The same is true for the content of PA and SA in some mitochondrial phospholipids of WEHI-164 and C6 cells, namely phosphatidylethanolamine and especially, cardiolipin (Table II). These phospholipids (and especially, their mixture) are more preferable substrates for phospholipase A_2 than phosphatydilcholine (Waite and Sisson, 1971), so it is possible that their hydrolysis can result in the level of PA and SA to exceed the treshold for the CsA-insensitive MPT. Whether this MPT will be followed by apoptosis or necrosis should depend on the dose of TNF used (Rath and Aggarwal, 1999).

No doubts, the composition of FFA pool in the mitochondrial membrane should be among the key factors, affecting the develoment of PA/Ca^{2+}-induced CsA-insensitive MPT. The data presented indicate that the saturated fatty acids amount to more that 50% of all FFA in mitochondria. Among unsaturated fatty acids, the main species are oleic and, at a smaller extent, linoleic acid (Table III). Other fatty acids (myristic, lauric, palmitoleic, linolenic, etc.) are presented in mitochondria in quantities, which are an order of magnitude lower than the quantity of main fatty acids—this should be taken into account when of interest is the effect of these fatty acids on the mitochondrial functions (Penzo *et al*., 2002). Turning back to the main FFA of the mitochondrial membrane (PA, SA, and olein acid), it should be noted that whereas PA and SA are effective in binding Ca^{2+} and triggering CsA-insensitive MPT, the oleic aicd does not bind Ca^{2+} (Mironova *et al*., 2001), does not alter the permeability of BLM and liposomes (Agafonov *et al*., 2003; Mironova *et al*., 2001), is not effeictive in the opening of CsA-insensitive MPT pore (Sultan and Sokolove, 2001a,b), and not always can induce apoptosis and necrosis of cells (Sparagna *et al*., 2000). Therefore, the question on the physiological relevance of CsA-insensitive MPT should be primarily attributed to PA and SA, with the attention drawn to the situations when their content in mitochondria increases substantially.

Comparing the composition of FFA from mitochondria and blood serum (Table III) suggests that the alterations in the FFA content of the mitochondrial membrane would relate to the general balance of FFA in an organism. This balance shows elevated FFA tissue levels upon various pathologies, such as diabetes (Gremlich *et al*., 1997), thyrotoxicosis (Harlan *et al*., 1963), adiposity (Zhou *et al*., 2000), and others. One example, concerning the relation between FFA and the development of myocardial ischemia has been considered in the present work. As follows from our data (Table IV), the heaviness of myocardial infarction correlates directly to the content of FFA in the blood serum. Taking into account the role of MPT in ischemia/reperfusion injury (Crompton, 2000; Halestrap *et al*., 1998) and the fact that in case of cardiomyocytes, PA (but not oleic acid) was shown to promote apoptosis (Sparagna *et al*., 2000), one should not ignore the involvement of PA/Ca^{2+}-induced CsA-insensitive MPT in the pathological processes in myocardium.

Thus, our data confirm a recent finding that besides the classical MPT pore, a CsA-insensitive pore also exists in mitochondria, with the mechanism of its opening being based on the formation of PA/Ca^{2+} complexes in the mitochondrial membrane. We think this pore can be equally important in the functional respect as the MPT pore, being responsible, in certain cases, for the PA-induced apoptosis. The studies on the mechanism of the formation and functioning of the PA/Ca^{2+}-induced MPT pore in mitochondria are in progress.

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