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Possible Mechanism for Formation and Regulation of the Palmitate-Induced Cyclosporin A-Insensitive Mitochondrial Pore

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Abstract—The mechanism of the palmitate-induced opening of the mitochondrial Ca^{2+}-dependent cyclosporin A (CsA)-insensitive pore was studied, as well as the influence on this process of well-known modulators of the CsA-sensitive Ca^{2+}-dependent pore. Palmitic acid, which can bind Ca^{2+} with high affinity, induced the CsA-insensitive swelling of mitochondria, whereas palmitoleic and 2-bromopalmitic acids, which have no such affinity for Ca^{2+}, failed to induce the pore opening. The palmitate-induced Ca^{2+}-dependent swelling of mitochondria was not affected by a well-known inhibitor of the CsA-sensitive pore (ADP) and an activator of this pore (inorganic phosphate, P_i). However, this swelling was inhibited by physiological concentrations of ATP ($[I]_{50}$ = 1.3 mM), but 100 μM ATP increased by 30% the rate of mitochondria swelling if Ca^{2+} had been added earlier. The effects of ATP (inhibition and activation) manifested themselves from different sides of the inner mitochondrial membrane. Mg^{2+} inhibited the palmitate-induced Ca^{2+}-dependent swelling of mitochondria with $[I]_{50}$ = 0.8 mM. It is concluded that palmitic acid induces the opening of the CsA-insensitive pore due to its ability for complexing with Ca^{2+}. A possible mechanism of the pore formation and the influence of some modulators on this process are discussed.

Key words: mitochondria, cyclosporin A-insensitive pore, palmitic acid, calcium

Calcium ions are well-known universal intracellular regulators. Their effects in the cell are thought to be mediated through special Ca^{2+}-binding proteins [1]. However, a new intracellular non-protein sensor for calcium ions was recently found in our laboratory—saturated long-chain fatty acids, such as palmitic and stearic acids. These fatty acids bind Ca^{2+} with affinity that is at least one or two orders of magnitude higher than the affinity of unsaturated fatty acids and other lipids and are comparable to the affinity for this ion of some proteins [2].

Free fatty acids are known to function in the cell as substrates of respiration [3], uncouplers of oxidative phosphorylation [4-6], secondary messengers [7], etc. They have also been shown to induce opening of the Ca^{2+}-dependent nonselective pore in the inner mitochondrial membrane [8]. However, the pattern of their involvement in this process is still not clear in detail. The opening of the Ca^{2+}-dependent mitochondrial pore in the presence of fatty acids was shown to be sensitive to the known pore inhibitor cyclosporin A (CsA) and also to carboxyatractylate and other inhibitors of adenylate translocator [8-11]. Thus, the pore induced by fatty acids was suggested to be a protein megachannel consisting of adenylate translocator, cyclophyllin D, and some other proteins [12]. The ability of fatty acids to induce the opening of the classical mitochondrial permeability transition (MPT) pore was explained by both their protonophore activity and direct interaction with adenylate translocator [9-11]. On the other hand, in the absence of P_i and other triggers of the Ca^{2+}-dependent pore, palmitic acid (and other long-chain saturated fatty acids) were recently found to induce opening of a pore insensitive to CsA [13-15]. This CsA-insensitive pore induced by saturated fatty acids is different from the MPT pore in some features. Thus, the palmitate-induced pore is short-living and can close spontaneously [13]. And we have shown that this is accompanied by recovery of $\Delta\psi$ on the inner mitochondrial membrane practically to the initial level [15].

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Based on these findings, we suggested that palmitic acid should induce opening of the mitochondrial pore by a mechanism different from the generally recognized one [10]. The supposed mechanism seemed to be based on a process that happens directly in the lipid bilayer [16]. We suggested that this process should be complexing of fatty acid with Ca^{2+} in the mitochondrial membrane. Thus, it was earlier found in our laboratory that nonspecific permeability of artificial lipid membranes (black-lipid membrane (BLM) and liposomes) was induced only by those fatty acids that have high affinity for Ca^{2+}, i.e., saturated fatty acids [2, 16].

To assess the contribution of Ca^{2+} complexing with palmitic acid to the opening of the CsA-insensitive pore in mitochondria, it was necessary to compare effects on this process of fatty acids with different abilities for binding Ca^{2+}. It was also necessary to determine the effect on the pore opening of well-known modulators of the MPT pore. Our attention was attracted by adenine nucleotides and Mg^{2+}, which seemed capable of influencing the binding of Ca^{2+} with palmitic acid. Thus, the purpose of this work was to continue studies on the mechanism of functioning of the CsA-insensitive Ca^{2+}-dependent mitochondrial pore induced by palmitic acid and compare features of this pore with the known features of the CsA-sensitive mitochondrial pore.

MATERIALS AND METHODS

Mitochondria were isolated from the liver of adult white rats with body weight of 220-250 g. The isolation medium contained 210 mM mannitol, 70 mM sucrose, 1 mM EDTA, 10 mM HEPES-KOH (pH 7.4). The liver was cooled in the isolation medium, washed free of blood, squeezed through a press with holes of ~1 mm diameter, and homogenized with a Teflon pestle in a glass Potter homogenizer (the ratio of tissue weight and isolation media was 1 : 8). The homogenate was centrifuged at 500 and 800*g* for 4 and 6 min, respectively. Mitochondria were precipitated for 20 min at 6000*g*. The mitochondrial precipitate was suspended in medium containing 210 mM mannitol, 70 mM sucrose, 0.05 mM EGTA, and 10 mM HEPES-KOH (pH 7.4) and centrifuged again for 20 min at 6000*g*. The resulting mitochondrial precipitate was resuspended in the isolation medium without EDTA and EGTA (0.1 ml per g liver). The resulting suspension of mitochondria contained 90-100 mg mitochondrial protein per ml. Concentration of the mitochondrial protein was determined by the Lowry method.

Mitochondrial swelling was recorded by changes in the optical density (at 540 nm) of the mitochondria suspension thermostatted at 25°C with constant stirring using an Ocean Optics USB 2000 spectrometric fiber-optic system (Ocean Optics Inc, USA). The incubation medium contained 210 mM mannitol, 70 mM sucrose, 5 mM succinic acid, 5 μM EGTA, 1 μM rotenone, and 10 mM HEPES-KOH (pH 7.4). The concentration of the mitochondrial protein in the cuvette was 0.4-0.5 mg/ml. In some experiments, the incubation medium was supplemented with oligomycin (2 μg/ml).

The rate of mitochondria swelling ($V_{max} = \Delta A_{540}$/min per mg protein) was calculated as the change in the optical density within the first 30 sec from beginning of the high-amplitude swelling. When modulators were used, the swelling rate was expressed in percent of the mean swelling rate recorded in a series of control experiments.

Unilamellar liposomes, prepared from azolectin and loaded with the fluorescent dye sulforhodamine B, were obtained by extrusion as described in [16]. The nonspecific permeability of the liposomal membrane was assessed by release of the dye from the liposomes by fluorimetry with a Kontron SFM25 spectrofluorimeter (Kontron Instruments, Italy) as described in [16]. The composition of the medium was as follows: 40 mM KCl, 5 μM EGTA, and 10 mM Tris-HCl (pH 8.5).

Chemicals. Sucrose, palmitic acid, succinic acid, ATP, ADP, atractyloside, EGTA, EDTA, CsA, rotenone, $MgCl_2$, HEPES, Tris, KH_2PO_4, and sulforhodamine B were from Sigma (USA); mannitol and $CaCl_2$ were from Merck (Germany).

RESULTS

In the present work the ability of fatty acids to induce opening of the mitochondrial pore was studied in dependence on their ability for binding Ca^{2+}. Palmitic acid (15 μM), which had a high affinity for Ca^{2+} [2], induced swelling of mitochondria in the presence of 30 μM free Ca^{2+} (Fig. 1, curve *1*). Fatty acids with a low affinity for Ca^{2+} (palmitoleic and 2-bromopalmitic acids) failed to induce the Ca^{2+}-dependent swelling of mitochondria (curves *3* and *4*). Palmitic acid induced high-amplitude mitochondrial swelling, which lacked a lag-period and was insensitive to the well-known inhibitor of the mitochondrial pore CsA (1 μM) (compare curves *1* and *2*).

Adenine nucleotides have been long known to inhibit opening of the CsA-sensitive mitochondrial pore [17]. We studied effects of adenine nucleotides (ADP and ATP) on opening of the CsA-insensitive pore induced by 15 μM palmitic acid and 30 μM Ca^{2+}. The nucleotides were added to the mitochondria 1 min before addition of inducers of the pore opening, palmitic acid and Ca^{2+}, for opening of the CsA-insensitive pore, and Ca^{2+} and KH_2PO_4 in the case of the CsA-sensitive (MPT) pore. The experiments were performed in the presence of oligomycin (2 μg/ml).

The addition of 2 mM ADP had practically no effect on the mitochondria swelling induced by palmitic acid and Ca^{2+} (Fig. 2a). This concentration of the nucleotide

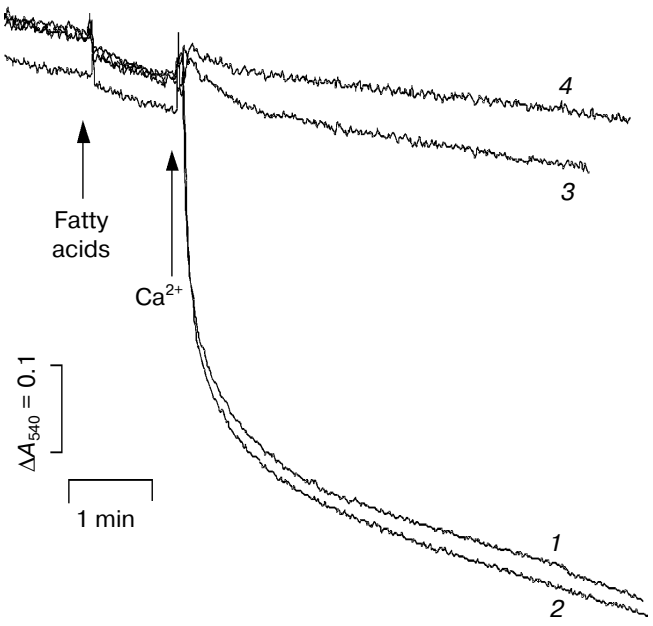

Fig. 1. Mitochondrial swelling induced by fatty acids in the absence (*1*, *3*, and *4*) and in the presence (*2*) of 1 μM CsA. The incubation medium contained 210 mM mannitol, 70 mM sucrose, 5 mM succinic acid, 5 μM EGTA, 1 μM rotenone, and 10 mM HEPES-KOH (pH 7.4). Additions: *1*, *2*) 15 μM palmitic acid and 30 μM Ca^{2+}; *3*) 15 μM palmitoleic acid and 30 μM Ca^{2+}; *4*) 15 μM 2-bromopalmitic acid and 30 μM Ca^{2+}.

decreased by only 15% the rate of mitochondria swelling, whereas 2 mM ADP completely inhibited the CsA-sensitive swelling of mitochondria induced by 50 μM Ca^{2+} in the presence of 1 mM KH_2PO_4 (Fig. 2c).

ATP is the much less effective inhibitor of the classical CsA-sensitive pore [8]. In our experiments, addition of 2 mM ATP into the incubation medium only temporarily prevented the Ca^{2+}-dependent swelling of mitochondria (the lag period was ~2 min) but did not lower its amplitude (Fig. 2d). But the same concentration of ATP completely inhibited the mitochondria swelling induced by addition of 15 μM palmitic acid and 30 μM Ca^{2+} (Fig. 2b). Half-maximal inhibition of the rate of the swelling induced by palmitic acid and Ca^{2+} occurred in the presence of 1.3 mM ATP (Fig. 3, curve *1*). Effects of GDP and GTP were similar to those of their phosphoadenine analogs (data not presented).

ATP also inhibited the release of sulforhodamine B from liposomes, which was induced by addition of 50 μM palmitic acid and 1 mM Ca^{2+} (table).

The mitochondrial swelling induced by palmitic acid and Ca^{2+} did not depend on order of the fatty acid and ion additions (data not presented). However, the order of palmitic acid and Ca^{2+} additions was significant for the effect of ATP. If the ATP-containing incubation medium was initially supplemented with palmitic acid and then with Ca^{2+} (direct order), the nucleotide in concentrations from 0.1 to 4 mM caused a pronounced inhi-

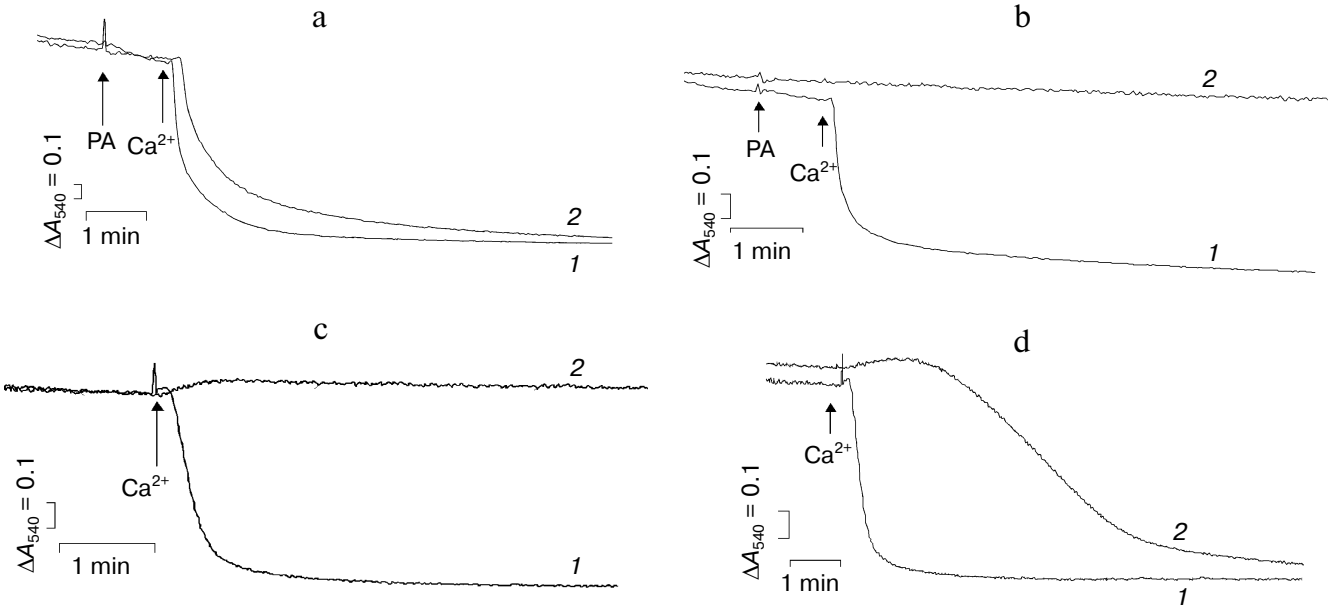

Fig. 2. The palmitate- (a, b) and phosphate-induced (c, d) Ca^{2+}-dependent swelling of mitochondria in the absence (*1*) and in the presence (*2*) of 2 mM ADP and ATP. The incubation medium was the same as in Fig. 1 but supplemented with oligomycin (2 μg/ml). Additions: a, b) 15 μM palmitic acid (PA), 30 μM Ca^{2+}, the incubation medium being supplemented with 1 μM CsA; c, d) 50 μM Ca^{2+}, the incubation medium being supplemented with 1 mM KH_2PO_4.

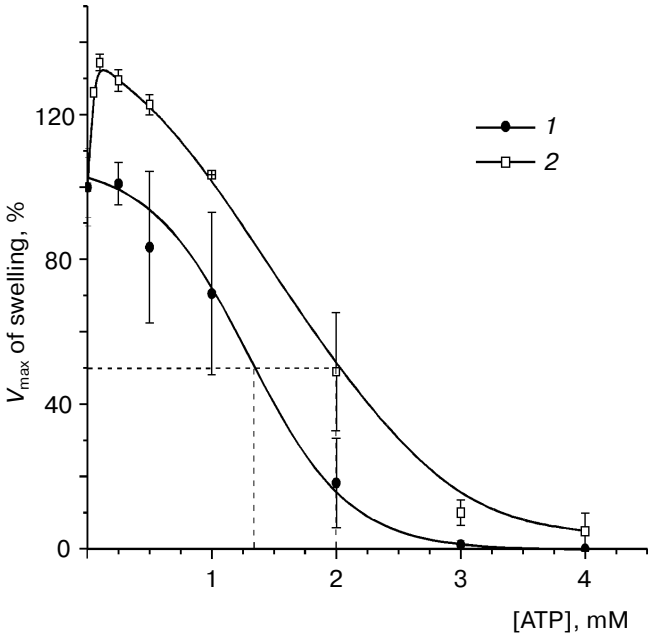

Fig. 3. Effect of ATP on the rate of mitochondrial swelling induced by addition of palmitic acid and Ca^{2+} in varied order. The incubation medium was the same as in Fig. 1 but supplemented with 1 μM CsA. The swelling of mitochondria was induced by: *1*) 15 μM palmitic acid, then 30 μM Ca^{2+}; *2*) 30 μM Ca^{2+}, then 15 μM palmitic acid. Mean values ± SD presented ($n = 4$).

bition (Fig. 3, curve *1*). If Ca^{2+} was added first and palmitic acid after (inverse order), the dependence of the swelling rate on the ATP concentration also changed (Fig. 3, curve *2*). In the latter case low concentrations of ATP increased the rate of mitochondria swelling (at 0.1 mM ATP this increase was ~30%). Higher concentrations of the nucleotide inhibited the pore opening ($[I]_{50}$ = 2 mM).

To determine what side of the inner mitochondrial membrane was affected by ATP, the entrance of the nucleotide into the mitochondrial matrix was prevented by the inhibitor of adenylate translocator atractyloside.

Atractyloside (5 μM) had practically no effect on the rate of mitochondria swelling for both the direct and inverse order of palmitic acid and Ca^{2+} additions. But atractyloside enhanced the inhibitory effect of 2 mM ATP on the mitochondria swelling induced by the direct order of the additions (palmitic acid → Ca^{2+}) (Fig. 4a). In this case, ATP did not enter the mitochondrial matrix and, as a result, its extramitochondrial concentration remained the same; therefore, it was suggested that the inhibitory effect of ATP should occur from the external side of the inner mitochondrial membrane. When inducers of the CsA-insensitive pore opening were added in inverse order (Ca^{2+}→ palmitic acid), addition of 5 μM atractyloside into the incubation medium decreased ~20% the stimulation of the rate of mitochondria swelling induced by 0.1 mM ATP (Fig. 4b). Thus, low concentrations of ATP increased the rate of mitochondria swelling acting from the matrix side of the inner mitochondrial membrane.

This effect of ATP was not mediated by its hydrolysis inside the mitochondria. The non-hydrolyzable ATP analog ATP-γ-S stimulated the swelling of mitochondria just like ATP itself (Fig. 5).

All these experiments were performed in magnesium-free medium. Mg^{2+} is a well-known inhibitor of the classical MPT pore. We earlier found that addition of Mg^{2+} into the incubation medium in the presence of palmitic acid and in the absence of Ca^{2+} failed to induce swelling of mitochondria (data not presented). However, addition of Mg^{2+} into the incubation medium inhibited the mitochondrial swelling induced by the subsequent addition of palmitic acid and Ca^{2+} ($[I]_{50}$ = 0.8 mM) (Fig. 6).

Release of sulforhodamine B from liposomes induced by addition of 50 μM palmitic acid and 1 mM Ca^{2+} in the presence of different concentrations of ATP (medium composition: 40 mM KCl, 5 μM EGTA, 10 mM Tris-HCl (pH 8.5); mean values ± SD are presented ($n = 3$))

[ATP], mM	Release of sulforhodamine B, % of total amount in liposomes
0.0	66.61 ± 3.01
1.5	39.09 ± 3.75
3.0	20.49 ± 4.55

DISCUSSION

At present, the Ca^{2+}-dependent nonspecific permeability of mitochondria is generally thought to be created via opening of the CsA-sensitive pore in the inner mitochondrial membrane [8]. However, palmitic acid was recently found to cause opening in mitochondria of another Ca^{2+}-dependent pore that is insensitive to CsA (Fig. 1, curves *1* and *2*) [13, 15]. The present work shows that the opening of this pore is determined by the earlier detected ability of palmitic acid for complexing with Ca^{2+} in the lipid bilayer, which was accompanied by increase in its permeability [2, 16]. Low concentrations of palmitic acid could induce the rapid high-amplitude Ca^{2+}-dependent CsA-insensitive swelling of mitochondria (Fig. 1). P_i (1 mM), which is an inducer of the CsA-sensitive pore, had no effect on the mitochondria swelling activated by palmitic acid and Ca^{2+} (data not presented). Unlike palmitic acid, palmitoleic acid (by our data, it practically does not bind Ca^{2+} [2]) could not induce the mitochondria swelling (Fig. 1, curves *2* and *3*). These results are in

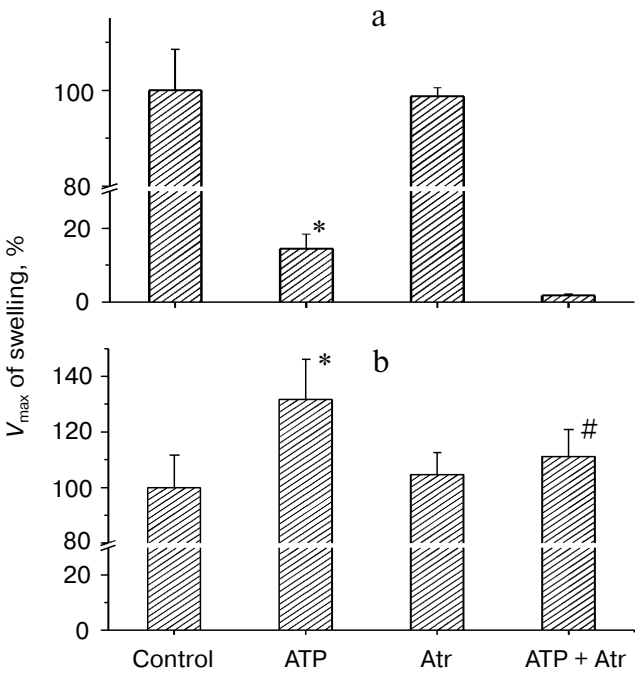

Fig. 4. Effect of atractyloside (Atr) on the ATP-induced changes in the rate of mitochondria swelling. The mitochondria swelling was induced by addition: a) 15 μM palmitic acid → 30 μM Ca^{2+}; b) 30 μM Ca^{2+} → 15 μM palmitic acid (control). The incubation medium was the same as in Fig. 1 but supplemented with 1 μM CsA and oligomycin (2 μg/ml). Additions: a) 2 mM ATP, 5 μM Atr; b) 0.1 mM ATP, 5 μM Atr. Mean values ± SD are presented (n = 6). * Differences between the control and experiment are significant at $p < 0.05$; # the difference with respect to the experiment with ATP is significant at $p < 0.05$.

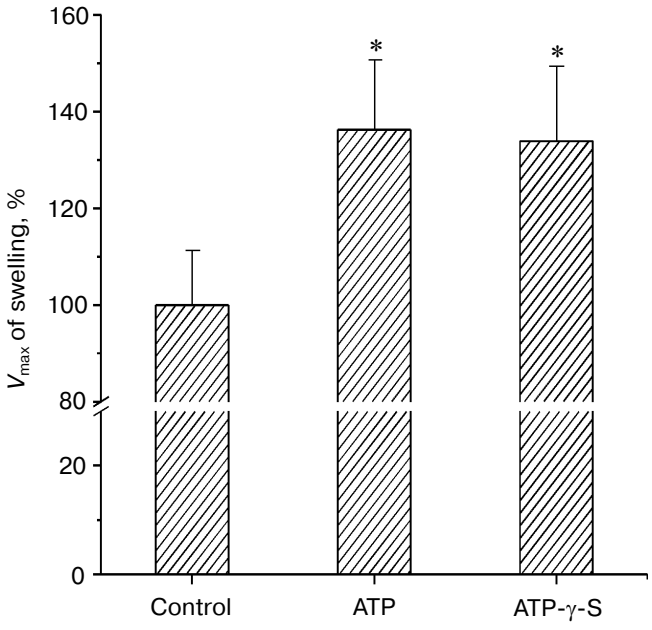

Fig. 5. Effects of ATP and ATP-γ-S on the CsA-insensitive mitochondrial swelling induced by 30 μM Ca^{2+} and 15 μM palmitic acid. The incubation medium was the same as in Fig. 1 but supplemented with 1 μM CsA. Additions: 0.1 mM ATP, 0.1 mM ATP-γ-S. Mean values ± SD are presented (n = 4-6). * Differences between the control and experiment are significant at $p < 0.05$.

agreement with our data on the effects of these fatty acids on the Ca^{2+}-dependent permeability of artificial lipid membranes (BLM and liposomes) [2, 16].

Substitution of the hydrogen atom with bromine at the second carbon atom in the acyl chain of palmitic acid (2-bromopalmitic acid) deprived palmitic acid of the ability for binding Ca^{2+} (data not presented) and induction of the pore formation (Fig. 1, curve *4*). Based on this series of experiments, the formation of the palmitate-induced pore was concluded to be based on complexing of the fatty acid with Ca^{2+} in the membrane.

The palmitate-induced mitochondrial pore is regulated by adenine nucleotides differently than the MPT pore. Adenylate translocator is thought to be the major component of the latter [8, 12], and this is responsible for its regulation by adenine nucleotides. ADP is a more powerful inhibitor of the mitochondrial pore than ATP. This was also confirmed by our studies (Fig. 2, c and d).

Another mechanism seems to underlie the opening of the palmitate-induced pore. First, ADP virtually does not inhibit the palmitate-induced swelling (Fig. 2a). Second, atractyloside, which itself induces the mitochon-

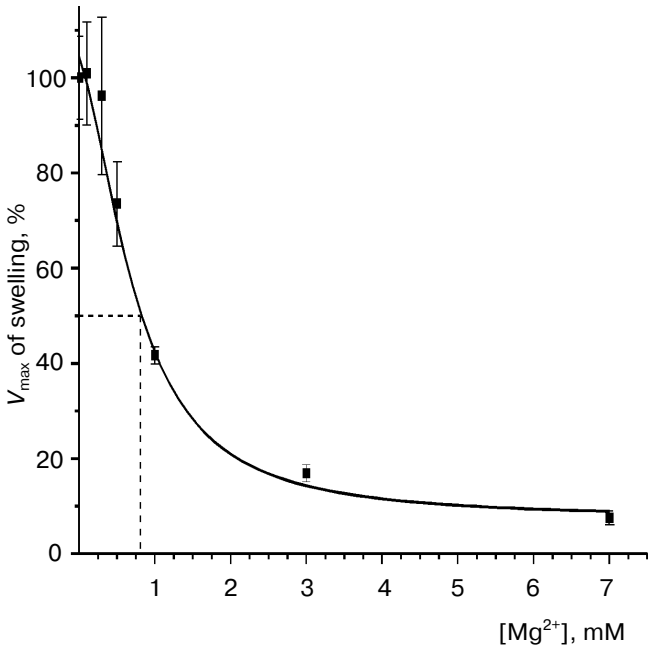

Fig. 6. Effect of Mg^{2+} on the rate of mitochondria swelling induced by 15 μM palmitic acid and 30 μM Ca^{2+}. The incubation medium the same as in Fig. 1 but supplemented with 1 μM CsA. Mean values ± SD are presented (n = 3).

drial CsA-sensitive pore via stabilization of the adenylate translocator in the "*c*" conformation [8, 18], has no effect on the mitochondria swelling induced by palmitic acid and Ca^{2+} (Fig. 4). Finally, the dual effect of ATP depending on the order of palmitic acid and Ca^{2+} additions (Figs. 3 and 4), which is manifested from different sides of the inner mitochondrial membrane, also cannot be explained by interaction of the nucleotide with the translocator. All this supports the idea of different mechanisms of formation of the pores under study.

It has already been mentioned that in our laboratory the CsA-insensitive pore induced by palmitic acid and Ca^{2+} was supposed to have a lipid nature [16]. Possibly, on binding to palmitic acid Ca^{2+} can cause its segregation into separate membrane domains and a subsequent crystallization of these domains. These processes should be accompanied by impairment of the balance between forces of lateral pressure in the inner and outer monolayers of the membrane. Relaxation of this tense state will result in rupture of the monolayers after which hydrophobic regions on the rupture edges will close with formation of a short-living hydrophilic lipid pore [16, 19].

Elimination of Ca^{2+} from the medium can inhibit opening of the mitochondrial pore. In our system, ATP can operate as a chelator of Ca^{2+} (K_d = 87 μM (pH 7.4)) [20]. This hypothesis was confirmed by our studies on liposomes when ATP inhibited the palmitate/Ca^{2+}-induced release of sulforhodamine B (table).

According to K_d, the affinity of ADP for Ca^{2+} is lower than the affinity of ATP (K_d of the ADP complex with Ca^{2+} is 180 μM (pH 7.4)) [20], and this appears to explain the inability of ADP to effectively inhibit the palmitate-induced mitochondrial swelling (Fig. 2a).

The stimulating effect of ATP (Fig. 3, curve *2*) on the palmitate-activated mitochondria swelling can manifest itself when the nucleotide is present in the mitochondrial matrix (Fig. 4b). This is not associated with ATP hydrolysis in mitochondria, which is proved by just the same effect of the non-hydrolyzable nucleotide analog ATP-γ-S (Fig. 5). The stimulating effect of ATP is likely to be based on providing in the mitochondrial matrix the locally increased concentration of Ca^{2+} bound to the nucleotide. This seems to increase the probability of the pore opening on the subsequent addition of fatty acid. The increase in the rate of mitochondrial swelling is likely to reflect this process. However, the exact mechanism of the stimulating effect is still not clear.

In the present work Mg^{2+} was shown to strongly inhibit the mitochondrial CsA-insensitive pore induced by palmitic acid and Ca^{2+} at $[I]_{50}$ = 0.8 mM (Fig. 6). This effect of magnesium ion may be explained by its inhibitory effect on the calcium uniporter [21] because the presence of Ca^{2+} in the mitochondrial matrix is indispensable for opening the pore induced by palmitic acid [13]. However, Mg^{2+}, due to a screening of the negative surface charge of the membrane, should be able to prevent the binding of palmitic acid with Ca^{2+}, as shown by us on model membranes [2, 22].

Palmitic acid was recently shown to be a natural inducer of apoptosis due to activation of the cytochrome *c* release from mitochondria [23]. It is likely that opening in mitochondria of the CsA-insensitive pore underlies the mechanism of the proapoptotic effect of palmitic acid. Oleic and 2-bromopalmitic acids, which do not bind Ca^{2+} and fail to induce the pore opening, do not promote apoptosis [23, 24].

Most likely, the palmitate-induced apoptosis can develop depending on amounts of palmitic acid and Ca^{2+} in mitochondria. Therefore, decrease in the amounts of the free fatty acid or Ca^{2+}, as well as decrease in the ability for complexing inside the membrane, is to significantly diminish the probability of the pore opening. In the present work, ATP and Mg^{2+} were shown to significantly decrease this ability.

Thus, the ability of palmitic acid to complex with Ca^{2+} inside the membrane is concluded to determine the opening in mitochondria of the CsA-insensitive pore.

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REFERENCES

1. Permyakov, E. A. (1993) *Calcium-Binding Proteins* [in Russian], Nauka, Moscow.
2. Mironova, G. D., Gateau-Roesch, O., Levrat, C., Gritsenko, E., Pavlov, E., Lazareva, A. V., Limarenko, E., Rey, P., Louisot, P., and Saris, N.-E. L. (2001) *J. Bioenerg. Biomembr.*, **33**, 319-331.
3. Lehninger, A. (1974) *Biochemistry: Molecular Fundamentals of the Cell Structure and Functions* [Russian translation], Mir, Moscow.
4. Dedukhova, V. J., Mokhova, E. N., Skulachev, V. P., and Starkov, A. A. (1991) *FEBS Lett.*, **295**, 51-54.
5. Skulachev, V. P. (1998) *Biochim. Biophys. Acta*, **1363**, 100-124.
6. Samartsev, V. N. (2000) *Biochemistry (Moscow)*, **65**, 991-1005.
7. Saltarelli, M., Yamada, K., and Coyle, J. (1990) *J. Neurosci.*, **10**, 62-72.
8. Zoratti, M., and Szabo, I. (1995) *Biochim. Biophys. Acta*, **1241**, 139-176.
9. Wieckowski, M., and Wojtczak, L. (1998) *FEBS Lett.*, **423**, 339-342.
10. Bodrova, M., Dedukhova, V., Samartsev, V., and Mokhova, E. (2000) *IUBMB Life*, **50**, 189-194.
11. Bodrova, M., Brailovskaya, I., Efron, G., Starkov, A., and Mokhova, E. (2003) *Biochemistry (Moscow)*, **68**, 391-398.
12. Halestrap, A., McStay, G., and Clarke, S. (2002) *Biochimie*, **84**, 153-166.
13. Sultan, A., and Sokolove, P. (2001) *Arch. Biochem. Biophys.*, **386**, 31-51.

14. Sultan, A., and Sokolove, P. (2001) *Arch. Biochem. Biophys.*, **386**, 52-61.
15. Mironova, G. D., Gritsenko, E., Gateau-Roesch, O., Levrat, C., Agafonov, A., Belosludtsev, K., France-Prigent, A., Muntean, D., Dubois, M., and Ovize, M. (2004) *J. Bioenerg. Biomembr.*, **36**, 171-178.
16. Agafonov, A., Gritsenko, E., Belosludtsev, K., Kovalev, A., Gateau-Roesch, O., Saris, N.-E., and Mironova, G. D. (2003) *Biochim. Biophys. Acta*, **1609**, 153-160.
17. Hunter, D., and Haworth, R. (1979) *Arch. Biochem. Biophys.*, **195**, 453-459.
18. Halestrap, A., and Davidson, A. (1990) *Biochem. J.*, **268**, 153-160.
19. Antonov, V., and Shevchenko, E. (1995) *Vestn. Ros. Akad. Med. Nauk*, **10**, 48-55.
20. DiStefano, B. V., and Neuman, W. F. (1953) *J. Biol. Chem.*, **200**, 759-763.
21. Gunter, T., and Pfeiffer, D. (1990) *Am. J. Physiol.*, **258**, C755-C786.
22. Gennis, P. (1997) *Biomembranes. Molecular Structure and Functions* [Russian translation], Mir, Moscow.
23. Sparagna, G., Hickson-Bick, D., Buja, L., and McMillin, J. (2000) *Am. J. Physiol. Heart Circ. Physiol.*, **279**, H2124-H2132.
24. Hardy, S., El-Assaad, W., Przybytkowski, E., Joly, E., Prentki, M., and Langelier, Y. (2003) *J. Biol. Chem.*, **278**, 31861-31870.