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Effect of ADP/ATP antiporter conformational state on the suppression of the nonspecific permeability of the inner mitochondrial membrane by cyclosporine A

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The influence of the conformational state of ADP/ATP antiporter on the efficiency of the inhibitory effect of cyclosporine A on the Ca^{2+}-induced nonspecific permeability of the inner mitochondrial membrane has been studied. Carboxyatractiloside, the inhibitor of ADP/ATP-antiporter, was shown to prevent the cyclosporine A-induced suppression of the nonspecific permeability. The carboxyatractiloside effect was displayed only in mitochondria depleted of adenine nucleotides. Bifunctional SH reagent, phenylarsine oxide, was also able to reverse the effect of cyclosporine A. The data are consistent with the suggestion that cyclosporine A causes suppression of the nonspecific permeability due to its effect on the ADP/ATP antiporter conformation.

Mitochondria; Nonspecific permeability; Cyclosporine A; ADP/ATP antiporter

1. INTRODUCTION

Accumulation of Ca^{2+} by mitochondria in the presence of P_i is known to result in the increase in the permeability of the inner mitochondrial membrane for substances with $M_r < 1500$ [1–3]. Current data [4–7] indicate that ADP/ATP antiporter is the key component which takes part in the induction of such a state of permeability. Stabilization of the nucleotide binding site of ADP/ATP antiporter on the matrix side of the inner membrane (m-state) by ADP or bongkrekic acid prevents the activation of the nonspecific permeability. In contrast, carboxyatractyloside, pyridoxal-5-phosphate and palmitoyl-CoA, stabilizing the nucleotide binding site on the cytoplasmic side of the inner membrane (c-state), exert the opposite effect [4–6]. It may be proposed that ADP/ATP antiporter either participates directly in the formation of the system, which provides nonspecific permeability, or regulates its activity. Recent studies on the reconstituted system, consisting of liposomes that carry enriched ADP/ATP antiporter, indicate that this carrier could be converted into a nonspecific transmembrane channel [7]. This argues in favour of the first version. However, the point of view that ADP/ATP antiporter directly takes part in the formation of a system providing the nonspecific permeability, is in a certain contradiction with data from laboratories of Pfeiffer [2] and Crompton [3]. These authors postulating the existence in the inner mitochondrial membrane of Ca^{2+}-dependent pore responsible for redistribution of low-M_r compounds show that the well known immunosuppressor, cyclosporine A, is apparently a specific inhibitor of this pore. At the same time, cyclosporine A has no apparent influence on the activity of ADP/ATP antiporter [8].

The aim of this study is to investigate the influence of the conformational state of ADP/ATP antiporter on the efficiency of suppression of the Ca^{2+}-induced nonspecific permeability transition by cyclosporine A.

2. MATERIALS AND METHODS

Rat liver mitochondria were isolated by differential centrifugation [9] in a medium containing 250 mM sucrose, 500 μM EDTA, 5 mM Hepes (pH 7.4). The final washing was performed in the same medium, but without EDTA. Protein concentration was determined by the biuret method using bovine serum albumin as a standard. TPP^+ concentration (TPP^+-selective electrode [10]) and mitochondrial swelling (light scattering at 660 nm) were registered simultaneously at the same measuring cell. $\Delta\Psi$ changes were evaluated by the TPP^+ distribution between the incubation medium and mitochondrial matrix. Mitochondria (0.5 mg/ml) were incubated at 26°C in a medium containing 10 mM succinate, 2 μM TPPCl, 2 μM rotenone, 10 mM H_3PO_4, 10 mM Mes-Tris (pH 7.4), plus a sufficient amount of sucrose to give a total osmotic strength of 300 mosM.

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Abbreviations: $\Delta\Psi$, mitochondrial inner-membrane potential; TPP^+, tetraphenylphosphonium; RLM, rat liver mitochondria; CATR, carboxyatractiloside; CSA, cyclosporine A; PhAsO, phenylarsine oxide

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3. RESULTS AND DISCUSSION

As follows from Fig. 1 (curve 3) the addition of Ca^{2+} to the mitochondrial suspension results in the transient drop of $\Delta\Psi$ due to the accumulation of these ions in the mitochondrial matrix. After some lag period a spontaneous decrease in $\Delta\Psi$ occurs. The second phase of the $\Delta\Psi$ decrease is thought to be caused by the opening of a nonspecific Ca^{2+}-dependent pore [1-3]. In accordance with data from [2,3,8] cyclosporine A completely prevents the development of the nonspecific permeability (Fig. 1, curve 1) while having no influence on the energy-dependent accumulation of Ca^{2+} ions and the ability of mitochondria to produce ATP (curve 2) [8]. As follows from curve 3, cyclosporine A is also able to restore the initial permeability of the inner mitochondrial membrane after practically complete mitochondrial deenergization (transition of almost entire mitochondrial population into the state of nonspecific permeability). Carboxyatractyloside is known to facilitate the transition into the state of nonspecific permeability, apparently as a result of the stabilization of ADP/ATP antiporter in c-state [4-6]. However, as follows from the comparison of curves 1 and 3, carboxyatractyloside is able to reverse the cyclosporine A effect in the only case when mitochondria have been initially passed through the state of nonspecific permeability. These results show that, first, the cyclosporine A effect is not due to the direct suppression of the nonspecific pore but is mediated through its effect on ADP/ATP antiporter, and secondly, for suppression by cyclosporine A of the nonspecific permeability increase, induced by stabilization of ADP/ATP antiporter in c-state, the presence of some low-M_r factor which has been lost after transition into the state of nonspecific permeability is required. Without this factor carboxyatractyloside provides the transition of ADP/ATP antiporter into c-state, thus inducing the nonspecific permeability even in the presence of cyclosporine A. The role of such a factor could be played by ADP, which is capable of stabilizing ADP/ATP antiporter in m-state. During the mitochondrial transition into the state of nonspecific permeability a release of ADP from the matrix occurs. ADP can be removed from the mitochondrial matrix by an exchange for PP_i. The ADP/PP_i exchange is catalyzed by ADP/ATP antiporter and is completely blocked by carboxyatractyloside [11,12].

As seen in Fig. 2 (curves 1,3) preincubation of mitochondria with PP_i lowers the efficiency of cyclosporine A to suppress the increase in the nonspecific permeability. It could be explained by reorientation of ADP/ATP antiporter from m- into c-state as a result of ADP loss by mitochondria. Carboxyatractyloside completely reverses the inhibitory effect of cyclosporine A. This is evident from the observed fast decrease in $\Delta\Psi$ (curve 2a) and the activation of the

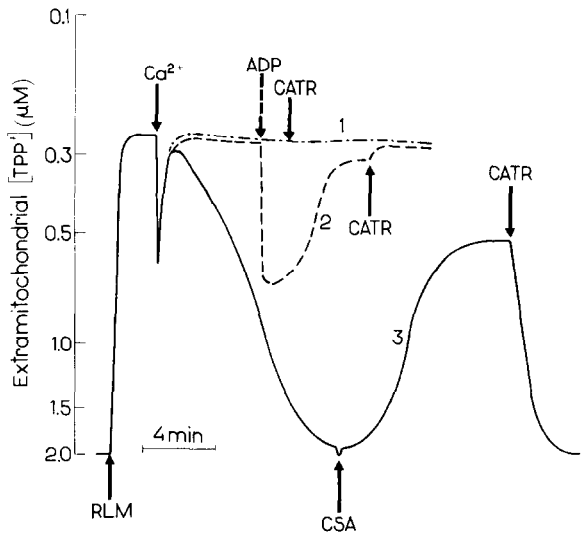

Fig. 1. The influence of cyclosporine A and carboxzyatractyloside on the Ca^{2+}-induced $\Delta\Psi$ decrease and oxidative phosphorylation. For conditions see section 2. (1,2) In the presence of 0.5 μM cyclosporine A; (3) control. Arrows show addition of $CaCl_2$ (20 nmol/mg protein), cyclosporine A, 250 μM ADP (curve 2), 5 μM carboxyatractyloside.

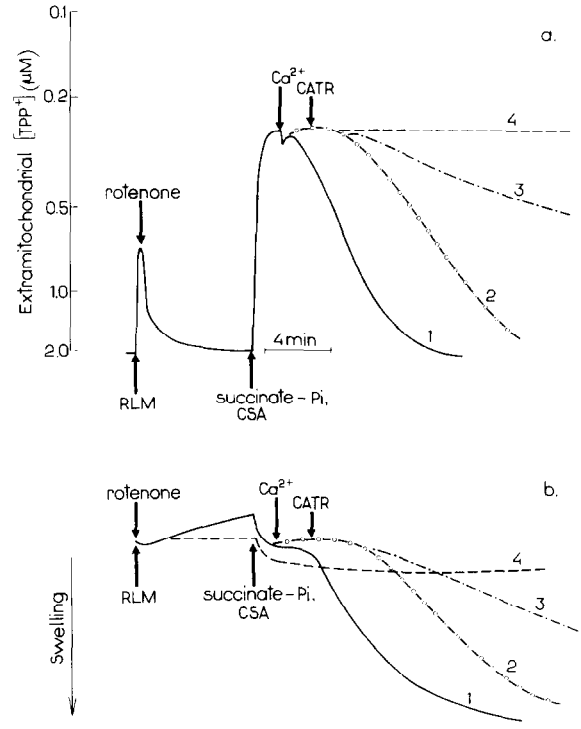

Fig. 2. The influence of cyclosporine A and carboxyatractyloside on the Ca^{2+}-induced $\Delta\Psi$ decrease (a) and high amplitude swelling (b) of mitochondria depleted of adenine nucleotides by preincubation with PP_i. For conditions see section 2. Succinate, P_i and rotenone were eliminated from the medium and 5 mM PP_i was added. (1) Control; (2-4) in the presence of 0.5 μM cyclosporine A. (3) In the absence of CATR; (2) CATR was added 2 min after $CaCl_2$; (4) 5 μM CATR was added to the incubation medium beforehand. Arrows show addition of $CaCl_2$ (25 nmol/mg protein), 5 μM carboxyatractyloside, 2 μM rotenone, 10 mM succinate, 5 mM P_i-Tris.

high amplitude mitochondrial swelling (curve 2b). At the same time, at the inhibition of the ADP/PP_i exchange by carboxyatractiloside addition simultaneously with mitochondria, cyclosporine A completely blocks the Ca^{2+}-induced nonspecific permeability (curves 4a,4b).

The data presented allow one to assume, first, that the cyclosporine A effect is mediated through its influence on ADP/ATP antiporter, and secondly, adenine nucleotides are obligatory components for displaying the effect of cyclosporine A. In this respect, of great interest are the data of Krämer [7] showing that modification of two SH groups of ADP/ATP antiporter, reconstituted into liposomes, results in the increase in ionic permeability of liposomal membranes.

On the basis of these data it is possible to predict that the suppression of the mitochondrial nonspecific permeability by cyclosporine A should be reversed by the reagents modifying SH groups, even without removing the endogenous adenine nucleotides. Really, as seen from Fig. 3, the addition of hydrophobic bifunctional SH reagent, PhAsO, completely reverses the suppression of the nonspecific permeability by cyclosporine A. The effect of PhAsO is explained by the cross-linking of two SH groups in a hydrophobic moiety of the membrane. This is proved by the complete removal of PhAsO effect by the hydrophobic monofunctional SH reagent, *N*-ethylmaleimide, but not by hydrophilic mersalyl (Fig. 4). The above fact excludes, in particular, the possibility of inhibition of the nonspecific permeability by *N*-ethylmaleimide through its binding with P_i/OH^- antiporter and the resulting blockade of P_i redistribution across the inner mitochondrial membrane. But it is worth noting that some difference in the activation of the nonspecific permeability by PhAsO is observed in the absence and presence of mersalyl (curves 1a,b; 2a,b). In the presence of mersalyl the phase of a partial mitochondrial de-energization, not accompanied by the high amplitude mitochondrial swelling, precedes the activation of the nonspecific permeability. One of the possible explanations for this fact may be that mersalyl modifies an access to factor B SH groups, of which the cross-linking by PhAsO induces the H^+ leakage (not coupled to ATP synthesis) through F_o of the ATP-synthase complex [13].

The results presented above show that, first, the suppression of the nonspecific permeability by cyclosporine A is mediated through its influence on

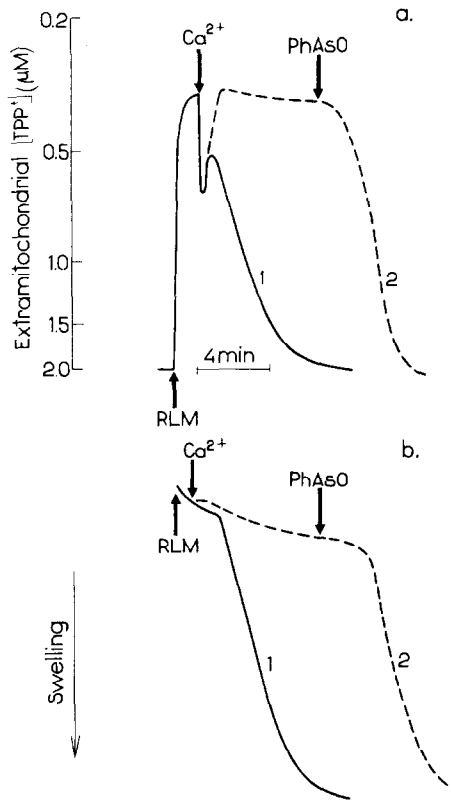

Fig. 3. The influence of PhAsO and cyclosporine A on the Ca^{2+}-induced $\Delta\Psi$ decrease (a) and high amplitude swelling of mitochondria (b). For conditions see section 2. (1) Control; (2) in the presence of 1 μM cyclosporine A. Arrows show the addition of $CaCl_2$ (30 nmol/mg protein), 15 μM PhAsO.

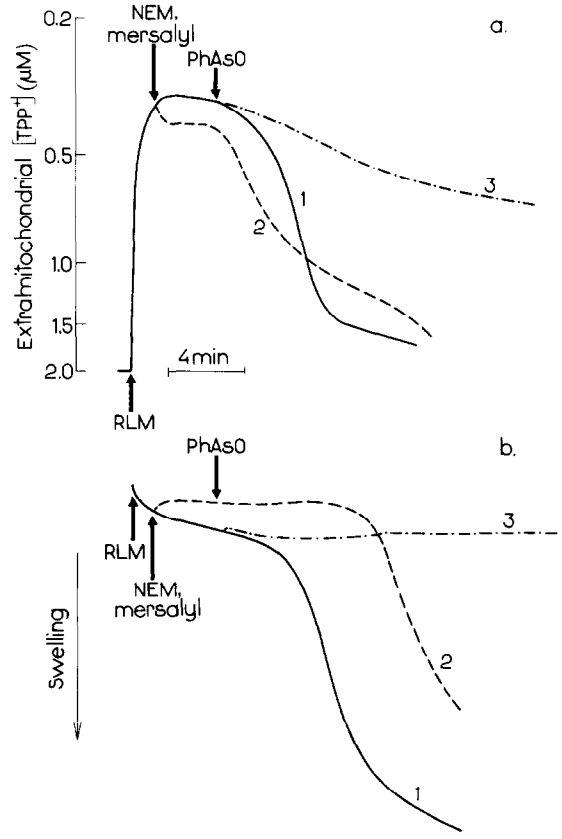

Fig. 4. The influence of *N*-ethylmaleimide and mersalyl on the PhAsO-induced $\Delta\Psi$ decrease (a) and high amplitude mitochondrial swelling (b). For conditions see section 2. (1-3) In the presence of 0.5 μM cyclosporine A; (1) control; (2) with mersalyl; (3) with *N*-ethylmaleimide (NEM). Arrows show the addition of 25 μM PhAsO, mersalyl (20 nmol/mg protein), 50 μM *N*-ethylmaleimide.

ADP/ATP antiporter; secondly, our data together with data of Krämer [7] allow to suppose that ADP/ATP antiporter itself is able under certain conditions to form a hydrophylic pore, providing the permeability of the inner mitochondrial membrane for low-M_r compounds. However, to confirm the last statement in experiments with reconstituted systems it is necessary to exclude the possibility that the system responsible for the nonspecific permeability does not co-purify with ADP/ATP antiporter because the usual impurity of isolated antiporter is about 25% [14].

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