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Effect of cyclosporine A and oligomycin on non-specific permeability of the inner mitochondrial membrane

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The effect of oligomycin and cyclosporine A on the induction of non-specific permeability of the inner mitochondrial membrane by Ca^{2+} was under study. Both oligomycin and cyclosporine A were able to prevent the activation of non-specific permeability, but cyclosporine A was the only agent which could restore initial permeability of the inner mitochondrial membrane. The effect of cyclosporine A was shown not to be mediated through redistribution of Ca^{2+} between different mitochondrial subpopulations

Mitochondria; Non-specific permeability; Cyclosporine A

1. INTRODUCTION

The experimental data available [1–4] allow one to postulate that besides very selective ion carriers within the inner mitochondrial membrane there is another carrier that provides non-specific redistribution of substances with $M_r < 1500$ (non-specific Ca^{2+}-dependent pore). The opening of the non-specific pore under the effect of Ca^{2+} ions or organic hydroperoxides induces mitochondrial uncoupling and redistribution of low M_r substances between the mitochondrial matrix and exterior [1–4]. It is possible to restore natural barrier properties of the inner mitochondrial membrane (pore closing) after ADP or Mg^{2+} addition or by Ca^{2+} chelating [5]. Oligomycin [6–8], dibucaine [9], buthylhydroxytoluene [6,7] and some other drugs are able to prevent pore opening. Recently a new specific inhibitor of Ca^{2+}-dependent pore, cyclosporine A (CSA), has been introduced [3,4]. CSA is shown to prevent pore opening while interacting with the pore itself or its regulating component [3,4].

In the present study the ability of CSA to induce the non-specific pore closing and restoration of initial barrier properties of the inner mitochondrial membrane was under investigation.

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Abbreviations: $\Delta\Psi$, mitochondrial inner-membrane potential; TPP^+, tetraphenylphosphonium; RLM, rat liver mitochondria; RR, Ruthenium red; CSA, cyclosporine A

2. MATERIALS AND METHODS

Rat liver mitochondria were isolated by differential centrifugation [10] in a medium containing 250 mM sucrose, 250 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 [11]) and mitochondrial swelling (light scattering at 660 nm) were registered simultaneously at the same measuring cell. The maximal mitochondrial swelling has been reached by addition of the pore-forming peptide, alamethicin K. Mitochondria (0.5 mg/ml) were incubated at 26°C in a medium containing 10 mM succinate, 2 μM TPP^+, 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.

3. RESULTS AND DISCUSSION

The addition of a limited amount of Ca^{2+} to mitochondria in the presence of inorganic phosphate transiently decreases $\Delta\Psi$ as a result of its accumulation within the mitochondrial matrix, and after some lag period a spontanous decrease in $\Delta\Psi$ (Fig. 1a, curve 3) and high-amplitude swelling occur (Fig. 1b, curve 3). The Ca^{2+}-induced $\Delta\Psi$ decrease and high-amplitude swelling are due to the opening of the non-specific Ca^{2+}-dependent pore, which results in the dissipation of ion gradients across the inner membrane and disturbance of the osmotic balance [1,12]. As shown in Fig. 1a,b (curves 1) and in accordance with the data of Broekemeier et al. [3] and Crompton et al. [4] the addition of CSA to the incubation medium completely supresses the Ca^{2+}-induced $\Delta\Psi$ decrease and high-amplitude mitochondrial swelling. A similar effect is demonstrated by the ATP synthase inhibitor, oligomy-

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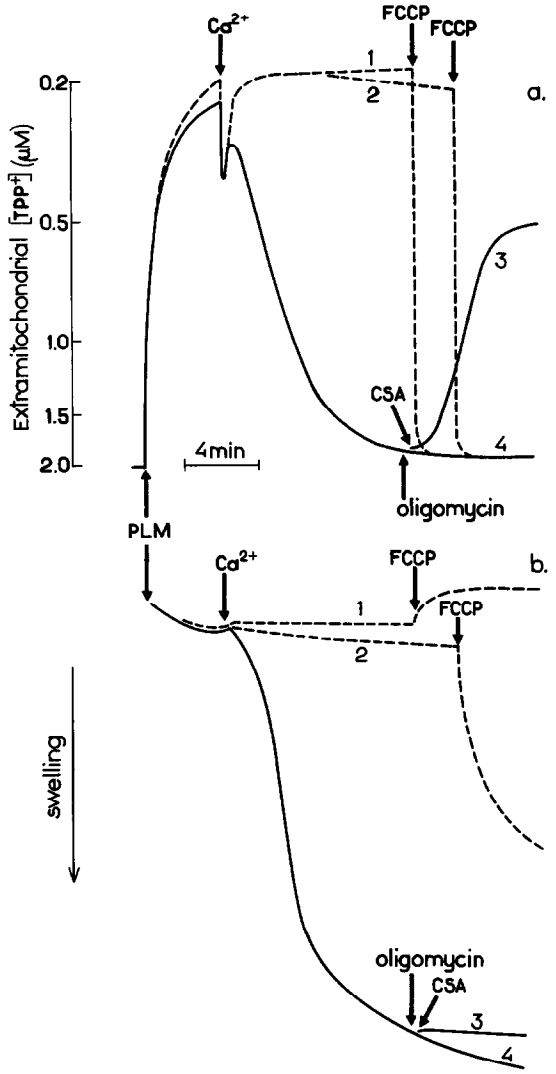

Fig. 1. The influence of CSA and oligomycin on the Ca^{2+}-induced $\Delta\Psi$ decrease (a) and high-amplitude mitochondrial swelling (b). For experimental conditions see Materials and Methods. 1, with 0.5 μM CSA; 2, with oligomycin (2 μg/mg protein); 3,4, control. Arrows show additions of $CaCl_2$ (30 nmol/mg protein), 0.5 μM CSA, oligomycin (2 μg/mg protein) and 2 μM FCCP.

cin (curves 2). Thus, both oligomycin and CSA are able to supress Ca^{2+}-induced non-specific permeability transition. From these two agents only CSA was able to restore $\Delta\Psi$ after practically complete de-energization of mitochondria (Fig. 1a, curves 3, 4). Thus, CSA cannot only prevent Ca^{2+}-induced non-specific permeability induction but also restore initial permeability of the mitochondrial membrane. These data are in agreement with those from [3,4] showing that the site of CSA action is exactly the pore itself or its regulating component. On the other hand, the oligomycin effect seems to be mediated through the influence on the electrogenic leakage of H^+ [13]. The release of the oligomycin effect by FCCP, the uncoupler, supports this suggestion (Fig. 1a,b, curve 2). As follows from Fig. 2, the ability of

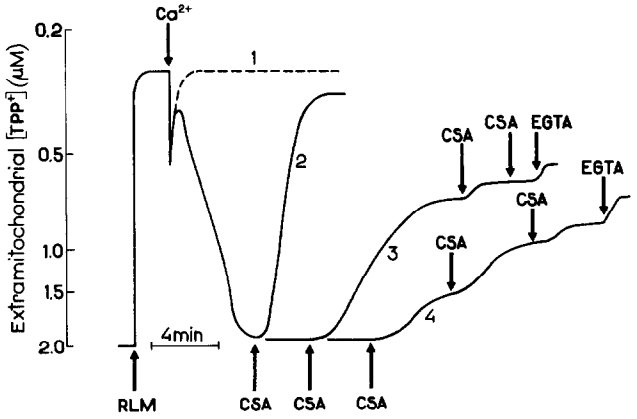

Fig. 2. The effect of the CSA addition time on the efficiency of mitochondrial $\Delta\Psi$ restoration. For experimental conditions see Materials and Methods. 1, 0.5 μM CSA was added simultaneously with mitochondria; 2–4, CSA was added after time intervals shown after the addition of Ca^{2+}. Arrows show addition of 0.5 μM CSA, $CaCl_2$ (20 nmol/mg protein), 500 μM EGTA.

CSA to raise $\Delta\Psi$ is diminishing with the increase in the time interval between Ca^{2+} and CSA additions. It could be due to the activation of phospholipase A_2 under experimental conditions and the distortion of barrier properties of lipid phase of the inner mitochondrial membrane as a result of accumulation of products of phospholipid hydrolysis [9]. However, it does not eliminate the possibility of time-dependent loss of sensitivity to CSA of the pore itself or its regulating component. In accordance with the data from [2,4,12] the mitochondrial de-energization coming from the rise of H^+ leakage across the inner mitochondrial membrane precedes the increase in non-specific permeability (pore opening). At this time the following transition of mitochondria into the state of non-specific permeability

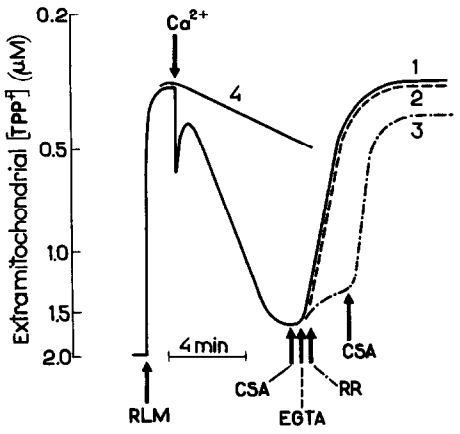

Fig. 3. The effect of RR and CSA on the Ca^{2+}-induced $\Delta\Psi$ decrease. For experimental conditions see Materials and Methods. 1-3, arrows show the addition of 0.5 μM CSA, 500 μM EGTA, RR (2 nmol/mg protein), $CaCl_2$ (20 nmol/mg protein). 4, RR was added to the incubation medium (2 nmol/protein). Arrow shows addition of $CaCl_2$ (20 nmol/mg protein).

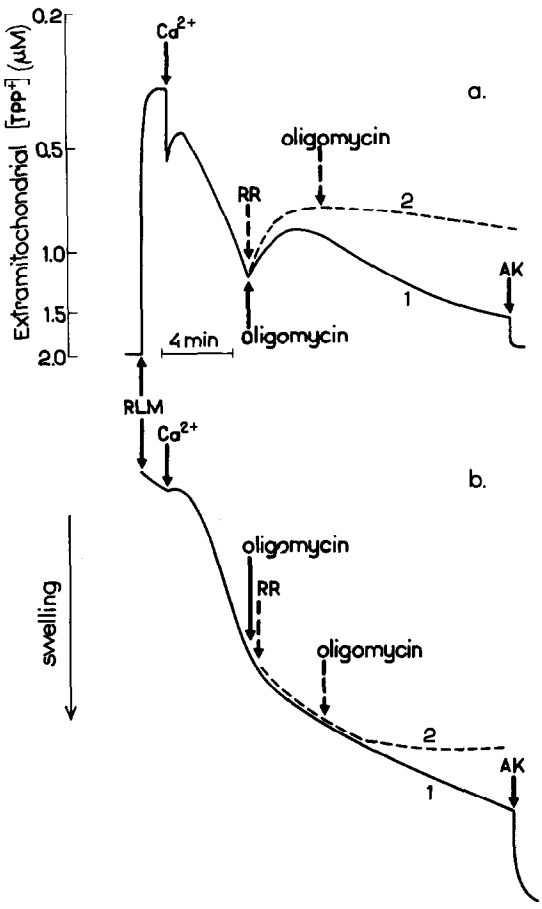

Fig. 4. The effect of RR and oligomycin on $\Delta\Psi$ (a) and high-amplitude mitochondrial swelling (b). For experimental conditions see Materials and Methods. Arrows show the additions of $CaCl_2$ (20 nmol/mg protein), RR (2 nmol/mg protein), oligomycin (2 μg/mg protein) and 7 μM alamethicin K (AK).

is being realized in a heterogenic manner. First, the rise of non-specific permeability in one mitochondrial subpopulation takes place. Then Ca^{2+} ions, released from this subpopulation are being accumulated by the next, previously more stable subpopulation, thus inducing its destabilization. During this transition both at the first step (rise in H^+ leakage) and at the final step (appearance of non-specific permeability), it is possible to observe mitochondrial oscillations between different states of permeability [2,12]. The process is completed by stabilizing the entire mitochondrial population in the state with an opened pore. In this case the addition of Ca^{2+} chelator, EGTA, but not of Ruthenium red, restores initial permeability of the inner mitochondrial membrane [2].

While analyzing the model presented above, it becomes clear that the restoration of $\Delta\Psi$ by CSA can have two alternative explanations. (1) CSA, binding with the non-specific pore or its regulating component, results in the pore closure. (2) CSA, binding with a small portion of mitochondrial population that did not pass into the state of non-specific permeability, sharply raises its ability to accumulate and retain Ca^{2+} ions. It must result in a sharp fall of Ca^{2+} concentration in the incubation medium and, as a sequence of that in the restoration of initial permeability in the mitochondrial subpopulation that are in the state with an opened pore (effect similar to the EGTA addition).

These two mechanisms could be distinguishable while using the specific inhibitor of the electrogenic system of Ca^{2+} transport, RR.

As seen from Fig. 3, RR addition in the concentration completely blocking Ca^{2+} accumulation (curve 4) to the practically de-energized mitochondria, as expected, induces an insignificant rise of $\Delta\Psi$ (curve 3) as compared with that after EGTA addition (curve 1). It gives the evidence that the mitochondrial population is mainly in the state with an opened pore. The following addition of CSA induces practically the same rise of $\Delta\Psi$ as the same addition but without RR (curve 2). That shows that CSA directly interacts with the system responsible for the non-specific permeability. At the same time we can predict that oligomycin being unable to close the pore and added at the initial steps of the process would inhibit the transition of mitochondrial population into the state of non-specific permeability. Partially the oligomycin effect should be mediated through the redistribution of Ca^{2+} between different mitochondrial subpopulations. Actually, as seen from Fig. 4, oligomycin added at the initial step of mitochondrial de-energization induces partial restoration of $\Delta\Psi$ in the absence of RR only (compare curves 1 and 2).

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