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Action of Cyclosporine on Mitochondrial Calcium Fluxes

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Abstract

Cyclosporine (Cys A) is a potent immunosuppressor used to reduce rejection in transplantation surgery. We studied its action upon mitochondrial functions: oxidative phosphorylation and Ca^{2+} movements through mitochondrial membrane. We show that Cys A exhibits an inhibitory effect upon mitochondrial respiration. This result is in good agreement with previous works and may be correlated with Cys A toxicity. The action of cyclosporine on calcium fluxes is more pronounced. Indeed it blocks mitochondrial calcium efflux and allows mitochondria to accumulate a large amount of calcium. If this effect occurs in the cell, it would induce a Ca^{2+} decrease in cytosol. This action might be correlated with the inhibitory effect of Cys A upon the mitogenic stimulation of T lymphocytes.

Key Words: Cyclosporine: calcium; mitochondria.

Introduction

Cyclosporine (Cys A) is a potent immunosuppressant widely used to reduce rejection in transplantation surgery (Borel *et al.*, 1977; Caine, 1979). The immunosuppressive mechanism of this drug is not completely known but its primary action is to antagonize T lymphocyte function (Morris, 1981). It has been shown that Cys A inhibits the lymphocyte activation induced either by mitogenic agents or by calcium ionophore A 23187 (Morris, 1981; Kay *et al.*, 1983).

Mitochondria play a role in dynamic calcium homeostasis of intracellular Ca^{2+} because of their great capacity for accumulating calcium (Fiskum,

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1984). So we studied the effect of Cyclosporine upon some mitochondrial functions: oxidative phosphorylation and Ca^{2+} movements. Because of the antagonizing effect of Cys A upon A 23187 action on lymphocytes, we also investigated its effect upon mitochondrial calcium recycling induced by the ionophore.

Materials and Methods

Reagents

Cyclosporine (Cys A) is a gift of Sandoz Ltd.; A 23187 is a Sigma product.

Rat Liver Mitochondria

Mitochondria were obtained from rat liver as described by Johnson and Lardy (1967). Mitochondrial protein was determined using Biorad protein assay (Biorad Laboratory).

Study of Respiration Control

Measurements of oxygen consumption were carried out at 25°C using a Clark microelectrode and a pH electrode fitted to a Gilson oxygraph. Mitochondria (1–2 mg of protein) were suspended in a 3.4-ml cell containing the respiratory medium (saccharose 0.25 M, KH_2PO_4 4 mM, rotenone 1 μM, pH 7.3, adjusted with KOH). The substrate used was sodium succinate 3 mM (final concentration). Oxidative phosphorylation was started by ADP addition (0.15 mM). Various doses of Cyclosporine (in ethanol) were added in a volume less than 50 μl at the beginning of the experiments. Controls were performed with the same quantities of ethanol.

Oxidation rates, respiratory control (RC), and P/O ratios were determined according to classical techniques (Chance and William, 1965). Oxidation rates were given as nmol of oxygen consumed per minute per milligram of mitochondrial protein. The respiratory control was the oxidation rate ratio between states 3 and 4. The P/O ratio gave the quantity of ADP molecules phosphorylated per oxygen atom consumed.

Calcium Fluxes

For these experiments the respiratory medium was supplemented with various doses of Ca^{2+} and Cys A. The quantities of mitochondria and reagents are specified in the figure legends. Calcium movements were followed by recording variations of extramitochondrial Ca^{2+} concentrations using a

specific Orion 9320 calcium electrode fitted to a PD2 Sefram recorder. These curves were used to quantitatively compare calcium storage in mitochondria under several experimental conditions. Oxygen consumption was recorded simultaneously.

The same experiments were performed in the presence of the calcium ionophore A 23187 (0.5 μg and 1 μg).

Measurements of Mitochondrial Membrane Potential

Membrane potential was measured using a tetraphenylphosphonium (TPP^+) electrode (Kamo *et al.*, 1979; Ducet *et al.*, 1983). The composition and the volume of the sample solution were identical to those used to study oxidative and Ca^{2+} transport activities except that a 10 μM concentration TPP^+ was added. Variations of membrane potential induced by Ca^{2+} addition (375 nmol) were recorded with and without various doses of Cyclosporine. The membrane potential was finally completely collapsed by addition of the uncoupler trifluorocarbonylcyanure (FCCP: 10^{-5} M final concentration) to appreciate its nul value.

Results

Respiratory Control

Table I shows the Cyclosporine action upon oxidative phosphorylation. Oxidation rates in state 3 and 4 and respiratory control are decreased. The results are the mean of three experiments performed with mitochondria from various preparations.

Calcium fluxes

Figure 1 shows a typical example of Cys A action on calcium fluxes with various concentrations of Cyclosporine (0.6 and 1.2 μM). With the Ca^{2+}

Table I. Action of Cyclosporine upon Respiratory Control (RC) and Oxidative Phosphorylation

Cys A (μM)	Oxidation rates		RC	P/O
	State 3	State 4		
0	97 ± 9	42 ± 5	2.3 ± 0.1	1.3 ± 0.1
4.8	77 ± 9	32 ± 3	2.4 ± 0.2	1.2 ± 0.1
12	56 ± 8	30 ± 3	1.9 ± 0.2	1.1 ± 0.1
24	46 ± 6	30 ± 2	1.5 ± 0.1	0.9 ± 0.1

[a]Experimental conditions are described in Materials and Methods (mean of three experiments ± SD).

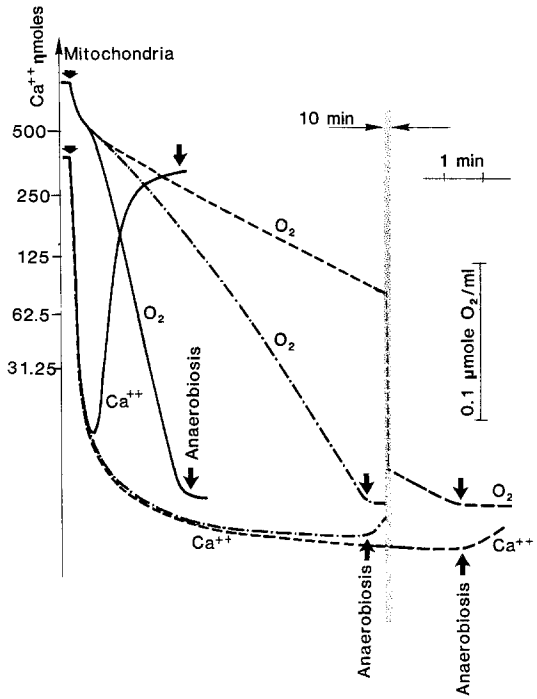

Fig. 1. Action of Cyclosporine on calcium transport into mitochondria. Mitochondria: 2 mg; Ca^{2+}: 375 nmol; succinate: 3 mM. Variation in calcium and O_2 concentration: (——) Without Cys A; (– · –) 0.6 μM Cys A; (– – –) 1.2 μM Cys A.

doses used, mitochondria are uncoupled and the calcium efflux is very fast. Cyclosporine counteracts this effect, the rate of O_2 consumption is drastically decreased and the calcium efflux occurs only after anaerobiosis. These experiments are performed in triplicate and give similar results.

Action of Ionophore A 23187

A 23187 classically hinders calcium storage in mitochondria, and Cys A does not correct this effect whatever its doses (0.48, 2.4, and 4.8 μM) (results not shown).

Membrane Potential Variations

As shown in Fig. 2, mitochondria in the presence of sodium succinate exhibit high membrane potential, which is drastically decreased by Ca^{2+} addition. In the absence of Cys A, mitochondria are unable to restore their potential, which has entirely collapsed (Fig. 2A). Addition of Cyclosporine in the respiratory medium allows mitochondria to recover their initial

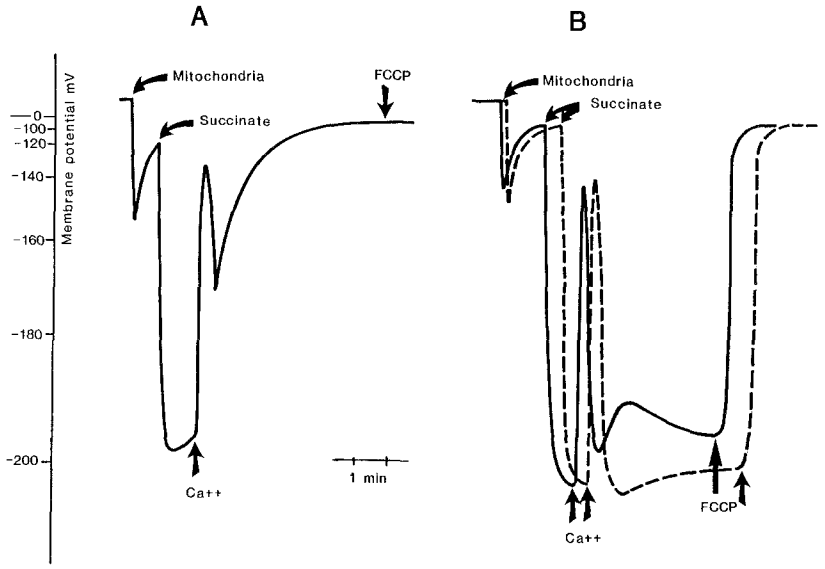

Fig. 2. Variation in mitochondrial membrane potential. Mitochondria: 2 mg; succinate: 3 mM; Ca^{2+}: 375 nmol. (A) Without Cys A; (B) With Cys A 0.12 μM (——) and 0.5 μM (– – –).

potential value after calcium addition (Fig. 2B). These experiments are perfomed in duplicate and give similar results.

Discussion

Cyclosporine affects mitochondrial respiration in doses higher than those used for Ca^{2+} flux assays. A decrease in respiration in both states 3 and 4 as well as some uncoupling is observed. (Table I). Our results are in good agreement with those in papers about renal toxicity of Cys A. Indeed, it has been shown that Cyclosporine induces a decrease of respiratory control and P/O ratios in rat kidney mitochondria (Jung and Pergande, 1985). Similarly, morphological changes in renal mitochondria occur in patients and rats treated with Cyclosporine (Verpooten *et al.*, 1986; Hay *et al.*, 1986), and a focal block of mitochondrial respiration is evoked to explain these alterations. Thus it seems likely that Cyclosporine exerts a toxic effect upon mitochondria whatever their origin. This hypothesis is corroborated by the decrease in ATP level in rat lymphocytes after cyclosporin treatment (Ferrero and Marni, 1985). Thus a part of the overall toxicity of the drug might be correlated with this action on mitochondria.

In the second part of our paper we showed that Cys A exerts a strong effect on Ca^{2+} fluxes through mitochondrial membrane. When Ca^{2+} is

added in high doses, mitochondria become unable to accumulate it and are totally uncoupled (Fig. 1). The addition of Cyclosporine restores the ability of mitochondria to store calcium and thus totally prevents damages induced by calcium. The concomitant measurements of membrane potential, a sensitive method used to appreciate the mitochondrial energized state, confirms this finding. Indeed, as indicated in Fig. 2, Cys A prevents the membrane potential collapse induced by Ca^{2+}. Our findings are in good agreement with the assumption of Wright *et al.* (1984) who suggest that Cyclosporine may act by blocking the mobilization of intracellular calcium stores. Moreover, Nicchitta *et al.* (1985) showed that Cys A increases the total calcium content in isolated hepatocytes but does not induce any apparent augmentation in cytosolic free Ca^{2+} level. This may, in part, be due to an increase of the mitochondrial Ca^{2+} pool as demonstrated by the action of FCCP on isolated hepatocytes. Our results, obtained directly with liver mitochondria, are in good accordance with the findings of Nicchitta *et al.* (1985).

In vivo, the main target of Cyclosporine is the lymphocyte, so our results on liver mitochondria must be used with caution when reported for lymphocyte mitochondria. But, as we said above, it seems that the action observed on liver mitochondria might be more general, so it is possible to think that the observed effects might occur, at least partially, in lymphocytes. In this case Cys A would induce a Ca^{2+} decrease in the cytosol. The first step of lymphocyte stimulation is an increase in free cytosolic calcium (Tsien *et al.*, 1982). Thus, our study would indicate that a part of the immunosuppressive activity of Cyclosporine A might be due to its capacity to increase calcium storage by mitochondria, thus diminishing the calcium-free form level in cytosol.

The last part of our study dealt with the effect of Cys A upon mitochondria damaged by ionophore A 23187, which hinders calcium transport into mitochondria. We showed that Cyclosporine A is unable to restore calcium storage. Thus, during ionophoric activation of lymphocytes, the action of Cyclosporine upon mitochondria would not account for the drug effect.

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