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INHIBITION BY CYCLOSPORINE A OF THE PROOXIDANT-INDUCED BUT NOT OF THE SODIUM-INDUCED CALCIUM RELEASE FROM RAT KIDNEY MITOCHONDRIA

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Abstract—The use of the immunosuppressive drug cyclosporine A (CSA) is restricted by its nephrotoxicity. Perturbation of Ca^{2+} homeostasis has been implicated in chemical toxicity. Mitochondria, a key regulator of Ca^{2+} homeostasis, may be a target of the drug. Here we show that CSA inhibits at low concentrations the prooxidant-induced but not the sodium-induced Ca^{2+} release from rat kidney mitochondria. CSA does not affect Ca^{2+} uptake by mitochondria. Inhibition of Ca^{2+} release is due to inhibition of intramitochondrial enzymatic hydrolysis of NAD^+ to ADP-ribose and nicotinamide. These findings suggest a very specific effect of CSA on mitochondrial Ca^{2+} release by which the drug interferes with cellular Ca^{2+} homeostasis. This is possibly the basis of CSA nephrotoxicity.

Ca^{2+} ions play a crucial role in many biological processes [1]. Therefore, a precise regulation of their concentration is required. Cellular Ca^{2+} homeostasis is achieved by the concerted action of membrane bound, ATP-driven Ca^{2+} pumps in both, plasma and endoplasmic reticular membrane, and by respiring mitochondria. Due to their relatively low Ca^{2+} affinity together with their very high Ca^{2+} storage capacity mitochondria may act as a safety device against a toxic increase of cytosolic Ca^{2+} [2, 3]. However, prolonged Ca^{2+} overloading of mitochondria is also dangerous since their energy metabolism, nucleic acid biosynthesis and other vital processes are regulated by intramitochondrial Ca^{2+}.

Mitochondria of probably all vertebrate tissues contain active Ca^{2+} transport systems in the inner membrane. Ca^{2+} uptake by energized mitochondria occurs via an electrophoretic Ca^{2+} uniporter which is driven by the mitochondrial membrane potential (negative inside) and can be inhibited, for example, by Ruthenium red. Release of Ca^{2+} occurs through two different pathways, one of which operates by an electroneutral $2Na^+/Ca^{2+}$ exchange mechanism (for reviews, see Refs 4 and 5). The other release mechanism is also electroneutral but exchanges H^+ and Ca^{2+}. It is not stimulated by Na^+ but can be activated by prooxidants (for review, see Ref. 3). The relative importance of the two release systems depends on the tissue origin of mitochondria. Whereas heart mitochondrial Ca^{2+} is controlled by (quasi) steady-state cycling mediated by the Ca^{2+} uniporter and the $2Na^+/Ca^{2+}$ carrier [4], liver mitochondrial cycling is mediated by the Ca^{2+} uniporter and the Na^+-independent, prooxidant-induced Ca^{2+} release system. The prooxidant-induced Ca^{2+} efflux is accompanied by pyridine nucleotide oxidation and hydrolysis, leaves mitochondria intact, and is, most likely, regulated by protein mono(ADP-ribosylation) (for review, see Ref. 3).

Cyclosporine A (CSA‡), a unadecapeptide of the fungus *Tolypocladium inflatum Gams*, has several pharmacological properties including antiparasitic and antimalarial activities and the potential for reversing multidrug resistance in tumors. Its clinically most relevant properties are, however, its unique immunosuppressive effects on certain immunocompetent cells, making it a powerful immunosuppressive agent in renal, liver, heart, and pancreatic transplantations. Its clinical use is limited by its nephrotoxicity in a high proportion of patients. Whereas much has been learned about the cellular biology of CSA [6, 7], the biochemical basis of the drug's action is not yet known and is currently being explored. We here report the specific inhibition by CSA of the prooxidant-induced Ca^{2+} release pathway from rat kidney mitochondria secondary to inhibition of the intramitochondrial hydrolysis of NAD^+ to nicotinamide and ADP-ribose. The Na^+-induced release pathway remains unaffected by CSA.

MATERIALS AND METHODS

Materials. Rotenone, HEPES and succinate were from the Sigma Chemical Co. (St Louis, MO, U.S.A.); arsenazo III, mannitol and sucrose were from Fluka (Buchs, Switzerland); tbh was from Merck (Darmstadt, Germany). CSA was a generous

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‡ Abbreviations: CSA, cyclosporine A; GSH, reduced glutathione; GSSG, glutathione disulfide; Hepes, 4-(2-hydroxymethyl)-1-piperazine-ethanesulfonic acid; MSH buffer, 210 mM mannitol, 70 mM sucrose, 5 mM Hepes, pH 7.4; tbh, *t*-butylhydroperoxide; RR, Ruthenium red, $[Ru_3O_2(NH_3)_{14}]Cl_6 \cdot 4H_2O$; arsenazo III, 2,2'-(1,8-dihydroxy-3,6 - disulfonaphthalene - 2,7-bis-azo)bis(benzene arsenic acid).

gift from Dr J. F. Borel, Sandoz AG (Basle, Switzerland).

Isolation of mitochondria. Kidney mitochondria from female Wistar rats (180–250 g, starved overnight and killed by decapitation) were isolated by conventional differential centrifugation using MSH buffer plus 1 mM EDTA as isolation medium. Mitochondria were washed twice in MSH buffer. The protein content of the mitochondrial suspension was determined by the biuret method with bovine serum albumin as standard.

Standard incubation procedure. Mitochondria at a concentration of 2 mg of protein/mL were incubated at 25° in 3 mL of MSH buffer under constant stirring and oxygenation. When appropriate, CSA was added at the indicated concentrations at the beginning of the incubation. Reduction of mitochondrial pyridine nucleotides and release of endogenous Ca^{2+} were induced by 5 μM rotenone. Mitochondria were then energized with 2.5 mM K^+-succinate. Where indicated, Ca^{2+} (8 nmol/mg of protein) was added and its uptake was allowed to proceed for the time shown in the figures. Finally, tbh or alloxan was added as indicated, preceded when appropriate by 2 nmol of RR/mg of protein.

Determination of Ca^{2+} uptake and release by mitochondria. Ca^{2+} movements across the inner mitochondrial membrane were monitored in a dual wavelength spectrophotometer (Aminco DW 2A) in MSH buffer in the presence of 50 μM arsenazo III at 685–675 nm [8].

Determination of mitochondrial pyridine nucleotides. Mitochondrial pyridine nucleotides were determined spectrophotometrically in a dual wavelength spectrophotometer at 340–370 nm.

Determination of mitochondrial GSH content. Mitochondrial GSH content was determined by the HPLC method of Reed *et al.* [9] with modifications as described by Fariss *et al.* [10].

RESULTS

Figure 1 shows the uptake of Ca^{2+} by energized rat kidney mitochondria followed by tbh-induced Ca^{2+} release. In contrast to liver mitochondria, the tbh-induced Ca^{2+} release from kidney mitochondria was not complete. Whereas CSA does not affect Ca^{2+} uptake, the tbh-induced Ca^{2+} release, which requires the concerted action of mitochondrial glutathione peroxidase (EC 1.11.1.9), glutathione reductase (EC 1.6.4.2) and the energy-linked transhydrogenase (EC 1.6.1.1) [3], is very sensitive to nanomolar CSA concentrations (Fig. 1). Fifty per cent inhibition of Ca^{2+} release was observed at about 50 nM CSA and at a concentration of 1 μM the inhibition was nearly 100%. When RR, an inhibitor of mitochondrial Ca^{2+} uptake, was added to mitochondria, "Ca^{2+} cycling" [11] was prevented and net Ca^{2+} release could therefore be observed spectrophotometrically. This is documented in Fig. 2. Even without addition of tbh (Fig. 2, Trace B) net Ca^{2+} release was observed which is in good agreement with previous findings for rat liver mitochondria [12]. This spontaneous Ca^{2+} release was partially inhibited by CSA (Fig. 2, Trace D). In accordance with previous findings for rat liver

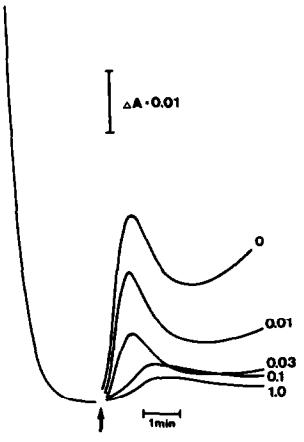


Fig. 1. tbh-Induced Ca^{2+} release from kidney mitochondria. In the presence of arsenazo III, kidney mitochondria were loaded with 8 nmol of Ca^{2+}/mg of protein. At the arrow, Ca^{2+} release was initiated by the addition of 100 μM tbh. Numbers next to the traces indicate CSA concentrations (μM).

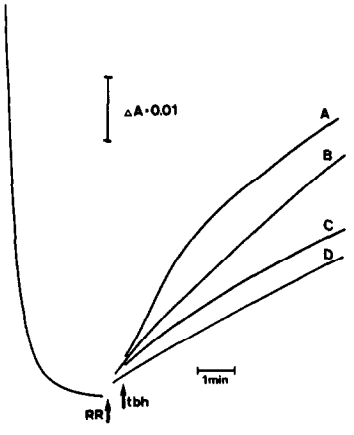
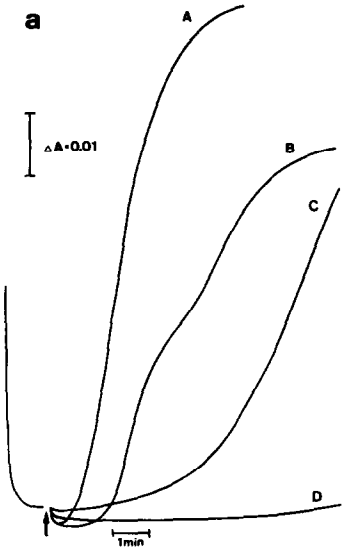


Fig. 2. tbh-Induced Ca^{2+} release from kidney mitochondria in the presence of RR. In the presence of arsenazo III, "spontaneous" Ca^{2+} efflux from kidney mitochondria was induced by RR (left arrow). At the right arrow (only traces A and C), 100 μM tbh was added. In trace A efflux induced by RR and tbh is monitored. Trace B shows efflux induced by RR alone (= control). Trace C: as trace A, but release inhibited by 1 μM CSA. Trace D: as trace B, but release inhibited by 1 μM CSA.

mitochondria [12, 13], tbh also caused in the presence of RR a stimulation of the Ca^{2+} efflux from kidney mitochondria (Fig. 2, Trace A). The tbh-stimulated efflux was likewise sensitive to CSA in the presence of RR (Fig. 2, Trace C). It should be noted that in the experiments of Fig. 2, mitochondria contained only the Ca^{2+} accumulated during the preparation (about 4 nmol of Ca^{2+}/mg of protein).

The prooxidant alloxan induces Ca^{2+} release from intact mouse [14] and rat [8] liver mitochondria. Unlike tbh, alloxan oxidizes intramitochondrial



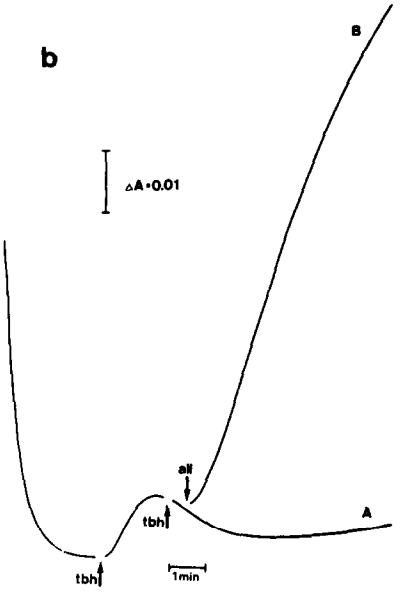


Fig. 3. Prooxidant-induced Ca^{2+} release from kidney mitochondria. In the presence of arsenazo III, kidney mitochondria were loaded with 8 nmol of Ca^{2+}/mg of protein. (a) At the arrow, Ca^{2+} efflux was initiated by the addition of 5 mM (traces A and B) or 1.4 mM (traces C and D) alloxan. The initial decrease in absorption which follows the addition of alloxan reflects absorption of alloxan itself. In trace A and C no CSA, in trace B and D 1 μM CSA was present. (b) At the two left arrows, 100 μM tbh were added (both traces) followed by addition of 1.4 mM alloxan in trace B.

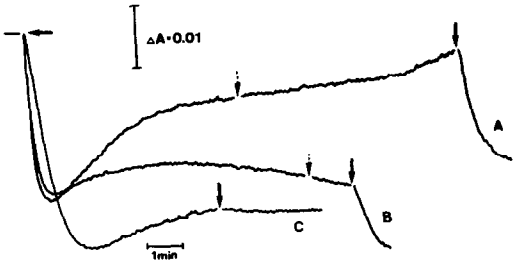


Fig. 4. Changes in the redox level of mitochondrial pyridine nucleotides. The redox level of mitochondrial pyridine nucleotides was monitored at 340–370 nm. Kidney mitochondria were energized with succinate and loaded with 8 nmol of Ca^{2+}/mg of protein. At the horizontal arrow, 100 μM tbh were added to the incubations monitored in traces A and B, and 1.4 mM alloxan were added in trace C, respectively. At the dashed arrows, further additions of 100 μM tbh and at the vertical arrows, further additions of 1.4 mM alloxan were made. The incubation monitored in trace A contained 1 μM CSA whereas the incubations monitored in traces B and C contained no CSA.

pyridine nucleotides in a predominantly non-enzymatic fashion [8]. Figure 3a shows that CSA completely inhibited Ca^{2+} efflux induced by 1.4 mM alloxan and clearly retarded efflux induced by 5 mM alloxan. In contrast to the findings with rat liver mitochondria [8], the extent of Ca^{2+} release from rat kidney mitochondria induced by tbh was clearly smaller than that induced by alloxan. Furthermore, alloxan could evoke Ca^{2+} efflux from kidney mitochondria which were not responsive to a second addition of tbh (Fig. 3b).

Pyridine nucleotide oxidation and hydrolysis are prerequisites for the prooxidant-induced mitochondrial Ca^{2+} efflux from mitochondria, with pyridine nucleotide hydrolysis requiring intramitochondrial Ca^{2+} [3]. Since tbh and alloxan cause pyridine nucleotide oxidation through different pathways (see above), the inhibition by CSA of Ca^{2+} efflux induced by either of the two compounds suggests that CSA does not interfere with the prooxidant-induced pyridine nucleotide oxidation. Indeed, CSA did not slow down the initial rate of oxidation of pyridine nucleotides (Fig. 4). CSA did, however, allow nearly complete re-reduction of pyridine nucleotides (Fig. 4, compare Traces A and B). The same held true when mitochondria were Ca^{2+}-depleted by pre-incubation with ethylene glycol bis(β-aminoethylether)-*N*,*N*,*N*′*N*′-tetraacetic acid (not shown), consistent with the notion that hydrolysis of the oxidized pyridine nucleotides requires Ca^{2+}. These findings suggest that CSA inhibits the hydrolysis of oxidized pyridine nucleotides. A second addition of tbh (Fig. 4, Trace A and B, dashed arrows) did not lead to further oxidation of pyridine nucleotides, whereas addition of alloxan caused a total oxidation of pyridine nucleotides (Fig. 4, vertical arrows and Trace C). Since alloxan is redox-cycled continuously in mitochondria at the expense of pyridine nucleotide oxidation [8] no re-reduction of pyridine nucleotides takes place. The absorption increase after oxidation of pyridine nucleotides by alloxan in Fig. 4, Trace C, did not reflect re-reduction since pyridine nucleotides are virtually completely hydrolysed under these conditions, but was caused by absorption of alloxan, a phenomenon described previously [8].

Figure 5 compares the sodium-induced with the tbh-induced Ca^{2+} efflux. Both compounds induced Ca^{2+} release from rat kidney mitochondria at a

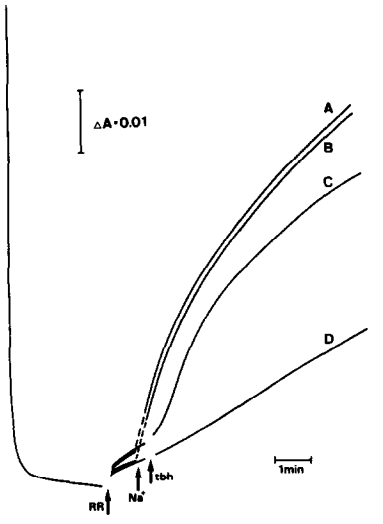


Fig. 5. Comparison of sodium- and prooxidant-induced Ca^{2+} release from kidney mitochondria. In the presence of arsenazo III, mitochondria were loaded with 8 nmol of Ca^{2+}/mg of protein. At the left arrow, 2 nmol of RR/mg of protein were added, followed by 10 mM sodium (middle arrow, traces A and B) or 100 μM tbh (right arrow, traces C and D). The incubations monitored in traces A and C contained no CSA; the incubations monitored in traces B and D were preincubated with 1 μM CSA.

similar rate and to a similar extent. However, sodium-induced efflux was totally insensitive to CSA, whereas tbh-induced efflux was almost completely inhibited by the drug.

Measurements of the GSH and GSSG content of rat liver and kidney mitochondria before addition of prooxidants revealed much higher GSH and GSSG levels in liver than in kidney mitochondria: 5.1 and 1.7 nmol GSH/mg of mitochondrial protein, and 0.34 and 0.11 nmol GSSG/mg of mitochondrial protein in liver and kidney mitochondria, respectively.

DISCUSSION

Prooxidants like tbh or alloxan induce Ca^{2+} release from intact mitochondria [8, 15]. Release requires both oxidation of pyridine nucleotides and hydrolysis to ADP-ribose and nicotinamide [3]. There is now convincing evidence that protein mono(ADP-ribosylation) regulates the prooxidant-induced Ca^{2+} release from intact respiring mitochondria [16]. CSA has been shown previously by Crompton *et al.* [17], Broekemeier *et al.* [18] and Richter *et al.* [19] to inhibit the hydroperoxide-induced Ca^{2+} release from heart mitochondria, the swelling of liver mitochondria and the prooxidant-induced Ca^{2+} efflux from intact liver mitochondria, respectively. In the present paper we show that CSA inhibits effectively the prooxidant-induced Ca^{2+} release from rat kidney mitochondria. It should be noted that also the "spontaneous" Ca^{2+} release is clearly slowed down by CSA. The residual release of Ca^{2+} in the presence of both, RR and CSA, (Fig. 2, Trace D) most likely reflects the normal leakiness of biological membranes.

The prooxidant-induced Ca^{2+} release operates by a Ca^{2+}/H^+ exchange [20]. It does not depend on the opening of a nonspecific pore of the inner mitochondrial membrane (Richter *et al.*, manuscript in preparation). We, therefore, suggest that CSA acts by preventing the initial reaction during protein ADP-ribosylation, i.e. pyridine nucleotide hydrolysis, as shown by the reversibility of pyridine nucleotide oxidation [21] in the presence of CSA. Therefore, the action of the drug is, as already discussed [13, 19], comparable to that of ATP and 4-hydroxynonenal which also inhibit pyridine nucleotide hydrolysis but is different from that of the other known inhibitor of prooxidant-induced Ca^{2+} release, *m*-iodobenzylguanidine, which inhibits presumably by competing in the ADP-ribosylation reaction [22].

Ca^{2+} can leave intact kidney mitochondria through two independent pathways (see introduction). CSA specifically inhibits the prooxidant-linked mitochondrial Ca^{2+} release but leaves the sodium-dependent pathway completely unaffected. This specificity will allow further characterization of these release pathways and may be important for a better understanding of CSA action at the molecular level.

Unlike Ca^{2+} efflux from rat liver mitochondria, efflux from kidney mitochondria is not complete when induced by tbh. Alloxan, however, induces complete release from both liver [8] and kidney mitochondria (Fig. 3b). This difference between liver and kidney mitochondria is due to the fact that oxidation of kidney mitochondrial pyridine nucleotides induced by tbh is incomplete in contrast to the total oxidation induced by alloxan (Fig. 4). In liver mitochondria, however, oxidation of pyridine nucleotides is complete with both tbh and alloxan [8]. The reason for these differences might be a limitation in the amount of GSH in kidney mitochondria because GSH as well as GSSG content in rat kidney mitochondria is three times lower than in rat liver mitochondria (see Results). This limitation is not important for the effect of alloxan which directly and non-enzymatically oxidizes mitochondrial pyridine nucleotides, i.e. does not engage the glutathione enzyme cascade [8].

The data presented here and in a previous publication [19] are in accordance with the increased content of the mitochondrial Ca^{2+} pool in isolated hepatocytes treated with CSA [23]. The data may also explain the hepatotoxicity [24] as well as the clinically important nephrotoxicity [25] of CSA. In general, excessive accumulation of Ca^{2+} is known to cause deterioration of mitochondrial functions [26]. Several mitochondrial dehydrogenases are regulated by micromolar and deactivated by higher concentrations of Ca^{2+} [26]. Large amounts of Ca^{2+} may also inhibit carbamoyl phosphate synthetase [27] and pyruvate carboxylase [28]. Furthermore, ATP [29] and RNA [30] syntheses are inhibited by increased amounts of mitochondrial Ca^{2+}. Therefore, a long term overloading of the mitochondria with Ca^{2+} due to an inhibition of the efflux pathway, e.g. by CSA, may compromise severely the cellular energy supply. The particular sensitivity to CSA of kidney cells can be explained by their low

mitochondrial glutathione content. CSA may deactivate mitochondrial dehydrogenases by enhancing the intramitochondrial Ca^{2+} concentration. The resulting insufficient electron supply would cause a drop in renal mitochondrial GSH to below a critical level, resulting in cell damage [31].

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REFERENCES

1. Campbell AK, *Intracellular Calcium, its Universal Role as Regulator*. John Wiley and Sons, New York, 1983.
2. Carafoli E, Intracellular calcium homeostasis. *Annu Rev Biochem* **56**: 395–433, 1987.
3. Richter C and Frei B, Ca^{2+} release from mitochondria induced by prooxidants. *Free Rad Biol Med* **4**: 365–375, 1988.
4. Nicholls DG, The regulation of extramitochondrial free calcium ion concentration by rat liver mitochondria. *Biochem J* **176**, 463–474, 1978.
5. Crompton M, The regulation of mitochondrial calcium transport in heart. *Curr Top Membr Transp* **25**: 231–276, 1985.
6. Borel JF, *Ciclosporin*. Karger, Basel, 1986.
7. Foxwell BMJ and Ryffel B, The mechanism of action of cyclosporine A. *Immunol All Clin North Am* **9**: 79–93, 1989.
8. Frei B, Winterhalter KH and Richter C, Mechanism of alloxan-induced calcium release from rat liver mitochondria. *J Biol Chem* **260**: 7394–7401, 1985.
9. Reed DJ, Babson JR, Beatty PW, Brodie AE, Ellis WW and Potter DW, High-performance liquid chromatography analysis of nanomole levels of glutathione, glutathione disulfide, and related thioles and disulfides. *Anal Biochem* **106**: 55–62, 1980.
10. Fariss MW, Olafsdottir K and Reed DJ, Extracellular calcium protects isolated rat hepatocytes from injury. *Biochem Biophys Res Commun* **121**: 102–110, 1984.
11. Carafoli E, The calcium cycle of mitochondria. *FEBS Lett* **104**: 1–5, 1979.
12. Baumhüter S and Richter C, The hydroperoxide-induced release of mitochondrial calcium occurs via a distinct pathway and leaves mitochondria intact. *FEBS Lett* **148**: 271–275, 1982.
13. Richter C and Meier P, Inhibition of pro-oxidant-induced mitochondrial pyridine nucleotide hydrolysis and calcium release by 4-hydroxynonenal. *Biochem J* **269**: 735–737, 1990.
14. Boquist L, Inhibition by dithioerytritol of alloxan induced efflux of Ca^{2+} and accompanying alterations in isolated liver mitochondria. *Biochem Int* **8**: 597–602, 1984.
15. Lötscher HR, Winterhalter KH, Carafoli E and Richter C, Hydroperoxide-induced loss of pyridine nucleotides and release of calcium from rat liver mitochondria. *J Biol Chem* **255**: 9325–9330, 1980.
16. Frei B and Richter C, Mono(ADP-ribosylation) in rat liver mitochondria. *Biochemistry* **27**: 529–535, 1988.
17. Crompton M, Ellinger H and Costi A, Inhibition by cyclosporine A of a Ca^{2+}-dependent pore in heart mitochondria activated by inorganic phosphate and oxidative stress. *Biochem J* **255**: 357–360, 1988.
18. Broekemeier KM, Dempsey ME and Pfeiffer DR, Cyclosporin A is a potent inhibitor of the inner membrane permeability transition in liver mitochondria. *J Biol Chem* **264**: 7826–7830, 1989.
19. Richter C, Theus M and Schlegel J, Cyclosporine A inhibits mitochondrial pyridine nucleotide hydrolysis and calcium release. *Biochem Pharmacol* **40**: 779–782, 1990.
20. Lötscher HR, Winterhalter KH, Carafoli E and Richter C, Hydroperoxides can modulate the redox state of pyridine nucleotides and the calcium balance in rat liver mitochondria. *Proc Natl Acad Sci USA* **76**: 4340–4344, 1979.
21. Richter C, The prooxidant-induced and spontaneous mitochondrial calcium release: inhibition by *meta*-iodobenzylguanidine (MIBG), a substrate for mono (ADP-ribosylation). *Free Rad Res Commun* **8**: 329–334, 1990.
22. Nicchita CV, Kamoun M and Williamson JR, Cyclosporine A augments receptor-mediated cellular Ca^{2+} fluxes in isolated hepatocytes. *J Biol Chem* **260**: 13613–13618, 1985.
23. Kassianides C, Nussenblatt R, Palestine AG, Mellow SD and Hoofnagle JH, Liver injury from cyclosporine-A. *Dig Dis Sci* **35**: 693–697, 1990.
24. Salaman JR, Cyclosporin in renal transplantation: a Guide to management. *Lancet* **ii**, 269–271, 1984.
25. Denton RM and McCormack JG, Calcium transport by mammalian mitochondria and its role in hormone action. *Am J Physiol* **248**: E543–E554, 1985.
26. Tuena De Gomez-Puyou M, Gavilanes M, Gomez-Puyou A and Ernster L, Control of activity states of heart mitochondrial ATPase. Role of the proton-motive force and Ca^{2+}. *Biochim Biophys Acta* **592**: 396–405, 1980.
27. Gaines G and Attardi G, Highly efficient RNA-synthesizing system that uses isolated human mitochondria: new initiation events and *in vivo*-like processing patterns. *Mol Cell Biol* **4**: 1605–1617, 1984.
28. Reed DJ, Glutathione: toxicological implications. *Annu Rev Pharmacol Toxicol* **30**: 603–631, 1990.
29. Mihatsch MJ, Olivieri W, Marbet U, Thiel G, Harder F and Zollinger HU, Giant mitochondria in renal tubular cells and cyclosporin A. *Lancet* **i**: 1162–1163, 1981.
30. Jung K and Pergande M, Influence of cyclosporin A on the respiration of isolated rat kidney mitochondria. *FEBS Lett* **183**: 167–169, 1985.
31. Jung K, Reinholdt C and Scholz P, Inhibited efficiency of kidney mitochondria isolated from rats treated with cyclosporin A. *Nephron* **45**: 43–45, 1987.