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Effects of the membrane potential upon the Ca^{2+}- and cumene hydroperoxide-induced permeabilization of the inner mitochondrial membrane

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A protonophore-induced $\Delta\Psi$ decrease in a 180–140 mV range causes an increase in the lag-period of Ca^{2+}-induced mitochondrial permeabilization but has little effect on the cumene hydroperoxide-induced permeability transition of mitochondria. Suppression of the non-specific permeability induction seems to be mediated by an increase in [ADP] in the mitochondrial matrix. A further decrease in $\Delta\Psi$ leads to additional suppression of the non-specific permeability as a result of a partial ruthenium red-sensitive efflux of the previously accumulated Ca^{2+}. On the other hand, complete dissipation of $\Delta\Psi$ causes immediate induction of the non-specific permeability. It is concluded that only complete dissipation of $\Delta\Psi$ caused by H^+ leakages may act as a trigger for non-specific permeability induction.

Mitochondrion; Non-specific permeability; Ca^{2+}

1. INTRODUCTION

Some compounds (so-called Ca^{2+}-releasing agents) are known to be capable of inducing non-specific permeability of the inner mitochondrial membrane for substances with M_r <1500 [1]. To explain this phenomenon it has been postulated that there exists a non-selective pore (the effective diameter is about 1.5 nm [2]) in the inner mitochondrial membrane. The mitochondrial de-energization caused by the rise of H^+ leakage through the inner mitochondrial membrane precedes the opening of the non-selective pore [3,4]. Earlier it was shown that a decrease in the $\Delta\Psi$ value may act as a trigger for the induction of the pore opening [5]. The experiment had been performed in the presence of the ATP-synthase inhibitor-oligomycin, thus excluding the possibility of the $\Delta\Psi$ effect on the non-specific permeability induction being mediated by a change in the ADP/ATP ratio in the mitochondrial matrix. However, it is still controversial whether partial de-energization of the inner mitochondrial membrane is sufficient for non-specific permeability induction, or whether complete dissipation of $\Delta\Psi$ is necessary. This question has been investigated in the present work.

Abbreviations: $\Delta\Psi$, mitochondrial inner-membrane potential; CATR, carboxyatractiloside; TPP^+, tetraphenylphosphonium; RLM, rat liver mitochondria; RR, Ruthenium red; CuOOH, cumene hydroperoxide; FCCP, carbonylcyanide-4-trifluoromethoxyphenylhydrazone.

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2. MATERIALS AND METHODS

Rat liver mitochondria were isolated by differential centrifugation [6] in a medium containing 250 mM sucrose, 500 μM EDTA and 5 mM HEPES (pH 7.4). The final washing was performed in the same medium, but without EDTA. The protein concentration was determined by the biuret method using bovine serum albumin as a standard. $\Delta\Psi$ changes were evaluated by the TPP^+ (TPP^+-selective electrode [7]) distribution between the incubation medium and the mitochondrial matrix. Ca^{2+} concentration was monitored with a Ca^{2+}-selective electrode [8]. High-amplitude mitochondrial swelling (light scattering at 666 nm) was recorded simultaneously in the same measuring cell. 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 for a total osmotic strength of 300 mOsm.

3. RESULTS AND DISCUSSION

Accumulation of Ca^{2+} by mitochondria in the presence of P_i gives rise to a time-dependent $\Delta\Psi$ decrease and high-amplitude mitochondrial swelling (Fig. 1a,b, curve 1). These processes reflect the activation of the non-specific permeability of the inner mitochondrial membrane [4]. A small decrease in the $\Delta\Psi$ value caused by the addition of protonophore FCCP after complete accumulation of Ca^{2+} into mitochondria considerably increases the lag-period of the non-specific permeability induction (Fig. 1a,b, curve 3). Such an increase period

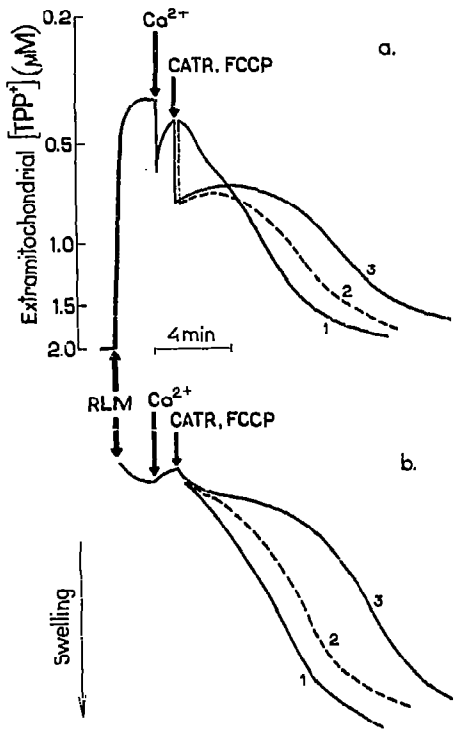

Fig. 1. Effect of FCCP and carboxyatractiloside on the Ca^{2+}-induced decrease of $\Delta\Psi$ (a) and high-amplitude mitochondrial swelling (b). For experimental conditions see Materials and Methods. (Trace 1) control; (trace 2) with FCCP and carboxyatractiloside; (trace 3) with FCCP. Arrows indicate additions of $CaCl_2$ (30 nmol/mg protein), 40 nM FCCP and 5 μM carboxyatractiloside (CATR).

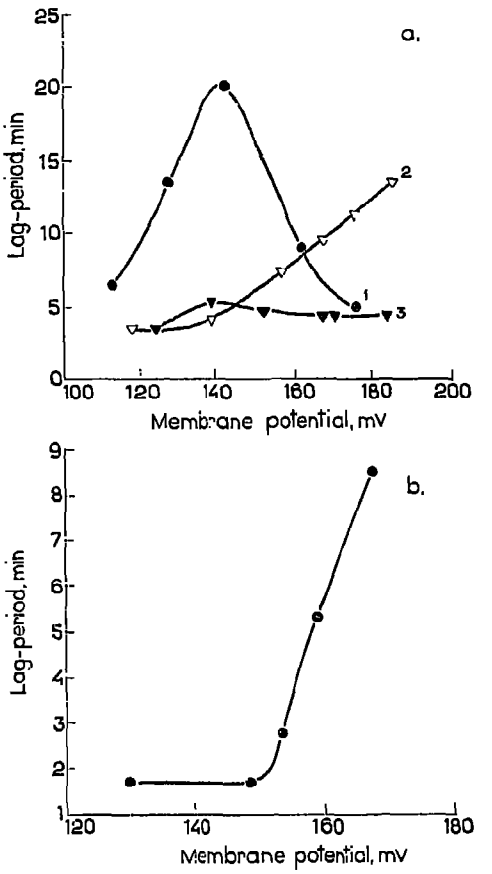

Fig. 2. Relationship between $\Delta\Psi$ and the lag-period of the non-specific permeability induction. For experimental conditions see Materials and Methods. $\Delta\Psi$ was varied by adding FCCP (10–100 nM) immediately after accumulation of added Ca^{2+}. (a1) Ca^{2+}-induced permeabilization (20 nmol Ca^{2+}/mg protein); (a2) CuOOH-induced permeabilization (150 μM CuOOH) in the presence of oligomycin (2 μg/mg protein); (a3) CuOOH-induced permeabilization (150 μM CuOOH); (b) Ca^{2+}-induced permeabilization (80 nmol Ca^{2+}/mg protein) in the presence of oligomycin (2 μg/ml protein).

is observed if $\Delta\Psi$ decreases to 140 mV (Fig. 2a, curve 1). A further decrease in $\Delta\Psi$ reduces the lag-period of non-specific permeability induction. In the case of CuOOH-induced non-specific permeability, $\Delta\Psi$ variations from 180–130 mV exert little, if any, effect on the lag-period (Fig. 2, curve 3). Suppression of the non-specific permeability by protonophore addition may be caused by a partial Ca^{2+} efflux from the mitochondrial matrix in response to the lowering of $\Delta\Psi$. However, Fig. 3a,b (curve 2) shows that the suppression of permeability transition by FCCP causes an increase in the amount of Ca^{2+} accumulated in the mitochondria before the induction of non-specific permeability. This is in agreement with the previously published data showing that a $\Delta\Psi$ decrease to 130 mV leads to a lowering of the steady-state $[Ca^{2+}]_o$ in a mitochondrial suspension [9,10]. On the other hand, the suppression of non-specific permeability by the protonophore may be due to the [ADP] increase in the mitochondrial matrix if $\Delta\Psi$ decreases. It is a well-known fact that ADP, in contrast to ATP, causes a closure of the non-specific pore [11]. The action of ADP is now attributed to its ability to stabilize the ADP/ATP antiporter (which plays a crucial role in the non-specific permeability regulation [12–14]) in the m-conformation, thus excluding mitochondrial permeabilization. As can be seen from Fig. 1, the ADP/ATP antiporter inhibitor, carboxyatractiloside, which is capable of removing ADP from the ADP/ATP antiporter nucleotide binding site and stabilizing the antiporter in the c-conformation, largely prevents the non-specific permeability suppression caused by a $\Delta\Psi$ decrease (Fig. 1a,b, curve 2). However, in the presence of the ATP synthase inhibitor, oligomycin, which prevents FCCP-induced changes in the ADP/ATP ratio caused by mitochondrial de-energization, only reduction of the lag-period of permeability transition is observed if $\Delta\Psi$ decreases in a 180–130 mV range. In the presence of oligomycin the uncoupler-caused decrease in the lag-period is observed both in the CuOOH- and in the Ca^{2+}-induced permeabilization (Fig. 2a, curve 2; Fig. 2b). Thus the non-specific permeability of the inner mitochondrial membrane may, on the one hand, be directly activated by a $\Delta\Psi$ decrease

and, on the other, be suppressed as a result of the increase in the mitochondrial matrix [ADP]. Yet the effect of $\Delta\Psi$ modulation on the lag-period of the CuOOH-induced permeabilization is less appreciable compared with the Ca^{2+}-induced one (Fig. 2a, curves 1,3). It may be related either to the CuOOH- induced increase in the Ca^{2+} sensitivity of the non-specific pore or to the decrease in the sensitivity to the inhibitory action of ADP. Although the $\Delta\Psi$ decrease to 109 mV exerts no substantial effect on the lag-period of the CuOOH-induced non-specific permeability activation (Fig. 4a, curves 1,2), a complete uncoupling of mitochondria by 1 μM of FCCP leads to virtually immediate permeabilization (Fig. 4b, curve 2). However, it should be noted that in this case the degree of mitochondrial swelling is drastically restricted. The addition of an intermediate FCCP concentration (0.5 μM), while leading to a $\Delta\Psi$ decrease below 109 mV but not to complete mitochondrial uncoupling, suppresses the high-amplitude swelling (Fig. 4a, curve 3). The suppression of mitochondrial permeabilization by high concentrations of FCCP must be perfectly clear in the light of the following data. Firstly, if $\Delta\Psi$ decreases below 130 mV, an efflux of the previously accumulated Ca^{2+} from the mitochondrial matrix [9,10] takes place. Secondly, according to Pfeiffer's model [4], the transition of a mitochondrial population into the state of non-specific permeability occurs in a heterogenic manner. Initially the non-specific permeability rises only in one of the mitochondrial subpopulations. Then Ca^{2+}, released from this subpopulation, is accumulated by the next, previously more stable subpopulation, whereby its destabilization is induced. Therefore, upon complete dissipation of $\Delta\Psi$ by the addition of 1 μM FCCP, rapid permeabilization of a less stable mitochondrial subpopulation occurs (Fig. 4b, curve 2). The velocity of this transition exceeds that of

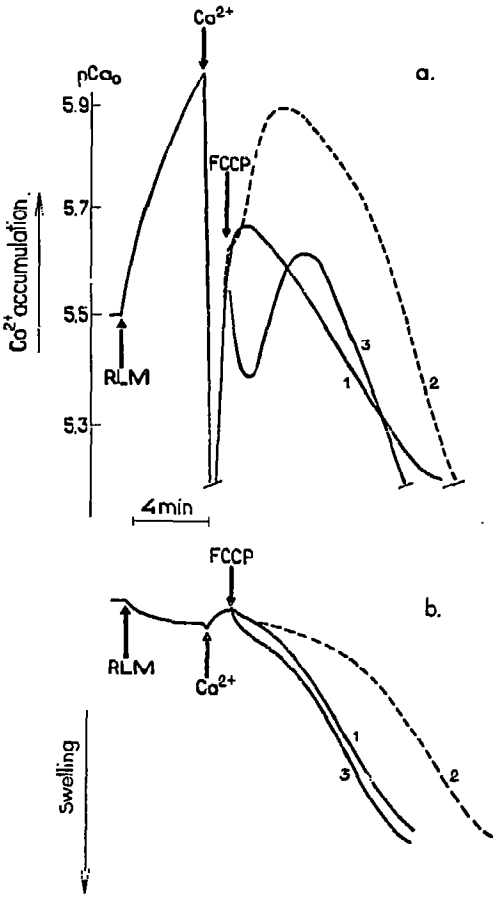

Fig. 3. Effect of FCCP on $[Ca^{2+}]_0$ (a) and high-amplitude mitochondrial swelling (b). For experimental conditions, see Materials and Methods. (Trace 1) control; (trace 2) with 80 nM FCCP; (trace 3) with 100 nM FCCP. Arrows indicate additions of Ca^{2+} (40 nmol/mg protein) and FCCP.

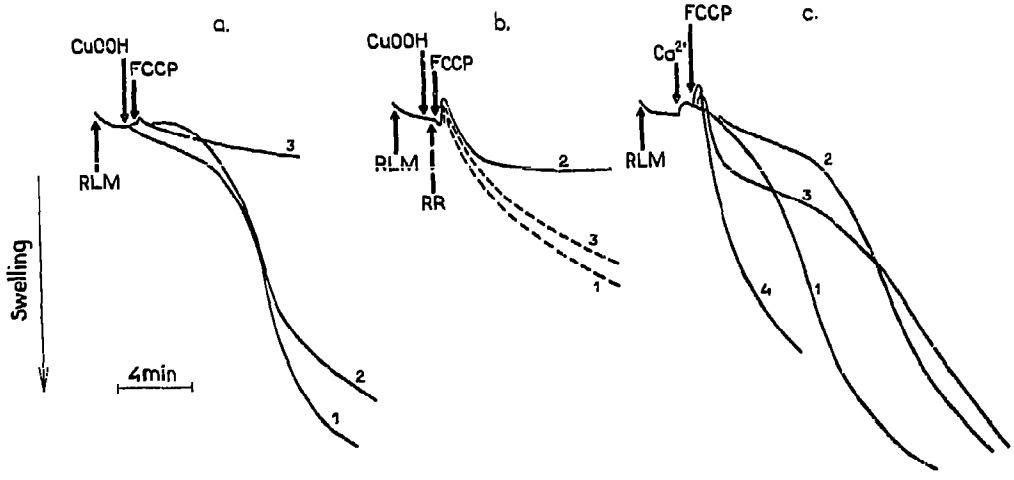

Fig. 4. Effect of FCCP and ruthenium red (RR) on the high-amplitude mitochondrial swelling induced by CuOOH (a,b) or Ca^{2+} (c). For experimental conditions, see Materials and Methods. (a1) control; (a2) with 100 nM FCCP; (a3) with 500 nM FCCP; (b1) with 500 nM FCCP and RR; (b2) with 1 μM FCCP; (b3) with 1 μM FCCP and RR; (c1) control; (c2) with 50 nM FCCP; (c3) with 100 nM FCCP; (c4) with 1 μM FCCP. Arrows indicate additions of Ca^{2+} (20 nmol/mg protein), 200 μM CuOOH, RR (3 μM) and FCCP.

the Ca^{2+} efflux through the ruthenium red (RR)-sensitive Ca^{2+} carrier. At the same time, permeabilization of the more stable subpopulation develops more slowly compared with the Ca^{2+} efflux from the mitochondrial matrix. If $\Delta\Psi$ is partially dissipated as a result of the addition of 0.5 μM of FCCP (Fig. 4a, curve 3), the decrease in $[Ca^{2+}]_{in}$ below the critical value in the whole mitochondrial population will be more rapid than is the permeability induction in the less stable subpopulation. Should this suggestion be correct, we may expect that the inhibition of the Ca^{2+} efflux from the mitochondrial matrix by RR should prevent the inhibitory action of high FCCP concentrations. Indeed, this has been observed in the experiments (Fig. 4b, curves 1,3). At the same time, as expected, the inhibitory action of high FCCP concentrations on the Ca^{2+}-induced permeabilization is less pronounced (Fig. 4c, curves 3,4). Thus the data obtained show that the increase in H^+ leakages of the inner mitochondrial membrane prior to permeabilization [3,4] may serve as a trigger for non-specific pore opening. The $\Delta\Psi$ decrease appears to result from the H^- leakage (non-coupled to ATP synthesis) through F_o of the ATP-synthase complex. This suggestion is supported by the ability of the exogenous protonophoric agent FCCP to overcome the effect of oligomycin on permeabilization (Fig. 2, [5,15]). Partial dissipation of $\Delta\Psi$ changes the ADP/ATP ratio in the mitochondrial matrix and induces an RR-sensitive efflux of Ca^{2+}, thus suppressing the induction of permeabilization. In this respect the non-specific permeability activation may be caused only by complete de-energization of the inner mitochondrial membrane.

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