Biochimica et Biophysica Acta 850 (1986) 41–48
Elsevier

BBA 42013

t-Butylhydroperoxide-induced Ca^{2+} efflux from liver mitochondria in the presence of physiological concentrations of Mg^{2+} and ATP

Celene Fernandes Bernardes, Lucia Pereira da Silva and Aníbal Eugênio Vercesi *

Departamento de Bioquímica, Instituto de Biologia, Universidade Estadual de Campinas, C.P. 6109, 13100 Campinas, São Paulo (Brazil)

(Received November 22nd, 1985)

Key words: Ca^{2+} efflux; NAD(P)H oxidation; Hydroperoxide; Ruthenium red; Acetate; (Rat liver mitochondria)

Isolated rat liver mitochondria, energized either by succinate oxidation or by ATP hydrolysis, present a transient increase in the rate of Ca^{2+} efflux concomitant to NAD(P)H oxidation by hydroperoxides when suspended in a medium containing 3 mM ATP, 4 mM Mg^{2+} and acetate as permeant anion. This is paralleled by an increase in the steady-state concentration of extramitochondrial Ca^{2+}, a small decrease in $\Delta\psi$ and an increase in the rate of respiration and mitochondrial swelling. With the exception of mitochondrial swelling all other events were found to be reversible. If Ca^{2+} cycling was prevented by ruthenium red, the changes in $\Delta\psi$, the rate of respiration and the extent of mitochondrial swelling were significantly diminished. In addition, there was no significant decrease in the content of mitochondrial pyridine nucleotides. Mitochondrial coupling was preserved after a cycle of Ca^{2+} release and re-uptake under these experimental conditions. It is concluded that hydroperoxide-induced Ca^{2+} efflux from intact mitochondria is related to the redox state of pyridine nucleotides.

Introduction

Energized liver mitochondria, particularly in the presence of membrane stabilizers such as ATP and Mg^{2+}, show the ability to buffer extramitochondrial Ca^{2+} at concentrations between 0.25 and 1.0 μM, depending on the medium composition [1–5]. This is the result of Ca^{2+} flux through two operative pathways: an uniporter that promotes an electrophoretic Ca^{2+} influx in response to the electrical membrane protential [6–11] and an electroneutral Ca^{2+} efflux through an antiport that exchanges one internal Ca^{2+} for two external H^+ [12–14], but see also Ref. 15. This model of Ca^{2+} transport in mitochondria was first suggested by Drahota et al. [16]. The concept of Ca^{2+} cycling through two independent pathways became established after experiments showing net Ca^{2+} efflux in the presence of ruthenium red, a specific inhibitor of the Ca^{2+} uniporter [17–19].

The steady-state extramitochondrial Ca^{2+} concentration can be altered in the presence of different agents which modify the rate of either Ca^{2+} influx or efflux [11,14,20]. It was first shown by Lehninger et al. [21] that Ca^{2+} efflux from isolated mitochondria could be stimulated by the oxidized state of mitochondrial pyridine nucleotides. This

* To whom correspondence should be addressed.

Abbreviations: *t*-BuOOH, *t*-butylhydroperoxide; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; TPP^+, tetraphenylphosphonium; FCCP, carbonyl cyanide *p*-trifluoromethoxyphenylhydrozone; $\Delta\psi$, transmembrane potential; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid.

0005-2728/86/$03.50 © 1986 Elsevier Science Publishers B.V. (Biomedical Division)

was subsequently confirmed by other laboratories not only in isolated mitochondria (Refs. 22–31, see also Vercesi, A.E, unpublished results), but also in intact cells [32] and perfused liver [33]. These findings resulted in a great controversy on the subject; some authors claimed that Ca^{2+} efflux stimulated by $NAD(P)^+$ was due mainly to non-specific increase in membrane permeability [22–27]. Others concluded that this release mechanism was independent of gross alterations in membrane permeability and could be of physiological relevance (Refs. 28–35, see also Vercesi, A.E., unpublished data). It has been shown that $NAD(P)^+$-induced Ca^{2+} release from mitochondria is accompanied by hydrolysis of oxidized pyridine nucleotides catalysed by an ATP-sensitive intramitochondrial NADase activated by Ca^{2+} [34,35]. This reaction produces nicotinamide, ADP-ribose and 5′-AMP [36]. It has been proposed that a posterior ADP-ribosylation of a protein in the inner mitochondrial membrane might allow Ca^{2+} release from mitochondria [36]. Inhibition of the NADase by ATP prevents both $NAD(P)^+$ hydrolysis and Ca^{2+} release from mitochondria [35,37].

It has also been shown that $NAD(P)^+$-induced Ca^{2+} release from mitochondria is involved in the perturbation of Ca^{2+} homeostasis and loss of cell viability during oxidative stress [38]. However, the inhibition by ATP would argue against the occurrence of this mechanism under physiological conditions [26]. In fact, it has been claimed [39] that NAD(P) oxidants cause a spontaneous Ca^{2+} discharge from mitochondria incubated in phosphate-containing media only in the absence of Mg^{2+} and adenine nucleotides.

In the present paper it is shown that NAD(P)H oxidation induced by *t*-butylhydroperoxide leads to a stimulation of Ca^{2+} efflux from mitochondria loaded with moderate Ca^{2+} concentrations even in the presence of physiological concentrations of ATP and Mg^{2+}, provided acetate instead of phosphate is used as permeant anion. Moreover, under these conditions, we found that $NAD(P)^+$-induced Ca^{2+} efflux is not attributed to mitochondrial uncoupling. A preliminary report of some of these findings has been presented [40].

Materials and Methods

Isolation of mitochondria. Adult female Wistar rats (4–6 months age) of 230–260 g body weight were fasted overnight and killed by decapitation. Liver mitochondria were isolated by homogenization in a medium containing 250 mM sucrose and 0.5 mM EGTA, followed by conventional differential centrifugation. The mitochondrial pellet was washed three times and then resuspended in the same medium without EGTA, at a concentration of 100 mg of protein per ml. The protein content was determined by the method of Kies and Murphy [41].

Standard incubation procedure. The experiments were carried out at 32°C in a basic medium containing 125 mM sucrose/65 mM KCl/3.0 mM Hepes buffer (pH 7.2)/4.0 mM Mg^{2+}/3.0 mM ATP/0.05% bovine serum albumin/4.0 μM rotenone/0.5 μg oligomycin per mg/10 mM K^+-acetate and 2.0 mM succinate. *t*-Butylhydroperoxide (40–80 μM) was added where indicated. The final Ca^{2+} loads given in Results and Discussion represent the sum of the endogenous and added Ca^{2+}. Other modifications of the basic medium are described in the figure legends.

Determination of Ca^{2+} movements and O_2 consumption. Ca^{2+} uptake and release were monitored using a Ca^{2+}-selective electrode (Radiometer, F2112 calcium selectrode). The response of the Ca^{2+} electrode was calibrated by addition of appropriated Ca^{2+}-EGTA buffer mixtures to the reaction medium [2,42]. Oxygen uptake was monitored with a Clark oxygen electrode (Yellow Springs Instruments Co.), in a 1 ml thermostatized chamber equipped with magnetic stirring. Total Ca^{2+} content of mitochondria was determined with an atomic absorption spectrophotometer (Perkin-Elmer 303).

Determination of the redox state of mitochondrial pyridine nucleotides. The redox state of NAD(P) was continuously measured fluorimetrically (366 → 450 nm) in an Aminco Bowman spectrofluorometer. In some experiments NADH and NADPH were determined enzymatically using lactate dehydrogenase and glutathione reductase as described by Beatrice et al. [43]. In this case, the fluorescence was compared to that obtained adding known amounts of NADH and NADPH as internal standards.

Determination of mitochondrial swelling. Mitochondrial swelling was estimated from light-scattering measurements at 520 nm, in an Aminco Bowman spectrofluorometer.

Measurement of mitochondrial transmembrane electrical potential ($\Delta\psi$). Mitochondria were incubated in the reaction medium containing 3 μM tetraphenylphosphonium (TPP^+). The concentration of TPP^+ in the extramitochondrial medium was continuously monitored with a TPP^+-selective electrode prepared in our laboratory according to Kamo et al. [44]. The mitochondrial membrane potential was then calculated assuming that the TPP^+ distribution between mitochondria and medium follows the Nernst equation [45].

Materials. Rotenone, oligomycin, ATP, NAD(P)H and *t*-butylhydroperoxide were obtained from Sigma; TPP^+ from Aldrich; all other reagents were commercial products of the highest available grade of purity.

Results and Discussion

Ca^{2+} flux and the redox state of mitochondrial pyridine nucleotides in the presence of t-butylhydroperoxide or acetoacetate

The experiment in Fig. 1 studies the Ca^{2+} flux in mitochondria exposed to either 1.0 mM acetoacetate or 50 μM hydroperoxide in the presence of 20 nmol Ca^{2+} per mg protein and physiological concentrations of ATP and Mg^{2+} [3]. It is claimed that in the presence of ATP and Mg^{2+} Ca^{2+} efflux is not stimulated by NAD(P)H oxidation because ATP and Mg^{2+} protect the 'damaging' effect of the oxidants on the membrane [26,27,39], or because ATP inhibits the activity of the mitochondrial NADase [35].

It can be observed that addition of mitochondria decreased the free Ca^{2+} concentration of the reaction medium until a steady state was achieved at an extramitochondrial Ca^{2+} concentration in the range of 0.75 μM in agreement with previous reports [1–4]. When 50 μM *t*-butylhydroperoxide was present in the medium prior to the inclusion of mitochondria the initial rate of Ca^{2+} uptake was identical to the control experiment but a transient increase in extramitochondrial Ca^{2+} concentration started just before the steady state was expected to be attained. Under such experimental

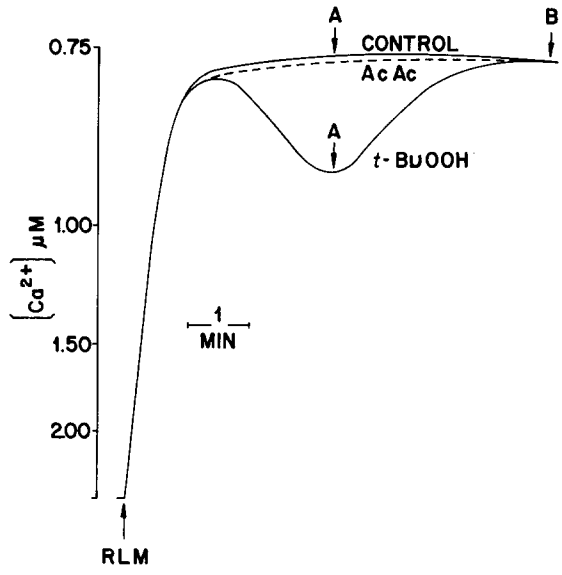

Fig. 1. Effect of *t*-butylhydroperoxide and acetoacetate (AcAc) on Ca^{2+} flux in liver mitochondria. Mitochondria (1.0 mg) were suspended in 1.0 ml of the basic medium (see Materials and Methods), in the absence of NAD(P)H oxidants (control), or in the presence of 50 μM *t*-butylhydroperoxide or 1.0 mM acetoacetate. RLM, rat liver mitochondria.

conditions this hydroperoxide-induced effect occurred only when acetate instead of phosphate was used as permeant anion. Acetoacetate at a concentration of 1.0 mM failed to induce net Ca^{2+} release, in agreement with previous experiments carried out in the presence of ATP [26]. To investigate at what extent pyridine nucleotides were oxidized under these conditions, samples were

TABLE I

CONTENT OF REDUCED PYRIDINE NUCLEOTIDES IN LIVER MITOCHONDRIA IN THE PRESENCE OF *t*-BUTYLHYDROPEROXIDE OR ACETOACETATE

Samples were taken from experiments described in Fig. 1, at the points indicated by the arrows and the reduced pyridine nucleotides were determined enzymatically as described in Materials and Methods.

Condition	Reduced pyridine nucleotides (nmol per mg protein)	
	NADH	NADPH
Control	3.52	4.75
t-BuOOH (A)	1.66	1.99
t-BuOOH (B)	3.37	4.08
Acetoacetate	1.59	4.73

taken from the reaction medium for enzymatic analysis where indicated by the arrows in Fig. 1. It can be seen in Table I that there was a marked decrease on both NADH and NADPH content in the samples taken during the hydroperoxide-induced increase in medium Ca^{2+} (A). Levels close to that of the control experiment were obtained in the samples taken after the Ca^{2+} re-uptake occurred (B). In the presence of acetoacetate, however, only NADH was significantly oxidized. These results are in agreement with our previous findings [29] that acetoacetate does not oxidize NADPH at high membrane potential, and therefore fails to stimulate Ca^{2+} efflux from mitochondria. On the other hand, hydroperoxide that oxidizes both NADH and NADPH stimulates Ca^{2+} efflux even in the presence of ATP and Mg^{2+}. The almost complete return of $NAD(P)^+$ to the reduced state observed when Ca^{2+} re-uptake was finished indicates that in the presence of ATP plus Mg^{2+}, Ca^{2+} efflux can occur in the absence of significant net loss of pyridine nucleotides, in agreement with recent results reported by Frei et al. [46]. The role of acetate on the process is still uncertain and may be explained, at least in part, on the basis of the findings of Bernardi et al. [47] showing that FCCP-induced Ca^{2+} efflux from rat liver mitochondria is increased by acetate and decreased by phosphate. Zoccarato and Nicholls [48] have also shown that in the presence of phosphate Ca^{2+} efflux from mitochondria is slower than in the presence of acetate due to intramitochondrial Ca^{2+} complexation by phosphate.

O_2 consumption, Ca^{2+} flux and swelling during hydroperoxide metabolism in liver mitochondria loaded with different Ca^{2+} concentrations: effect of ATP and Mg^{2+}

Some authors studying the mechanism of $NAD(P)^+$-induced Ca^{2+} efflux from mitochondria loaded with massive amounts of Ca^{2+} reported that Ca^{2+} release was preceded by a large-amplitude mitochondrial swelling and uncoupling [22–27]. This led us to study these parameters in liver mitochondria loaded with different Ca^{2+} concentrations, under the conditions of Fig. 1, where Ca^{2+} release was completely reversible. The oxidation of mitochondrial pyridine nucleotides was promoted by 50 μM *t*-butylhydroperoxide and

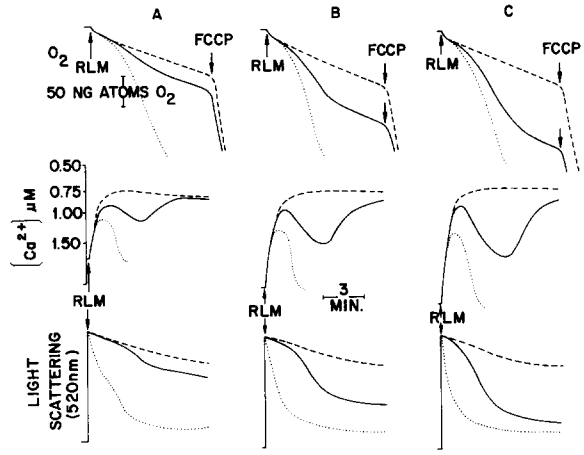

Fig. 2. Effect of *t*-butylhydroperoxide on Ca^{2+} flux, the rate of respiration and swelling in mitochondria loaded with different Ca^{2+} concentrations. Mitochondria (1.0 mg) were suspended in the basic medium in the presence (solid line) or absence (dashed line) of 50 μM *t*-butylhydroperoxide. The dotted lines show the effect of 50 μM *t*-butylhydroperoxide in mitochondria suspended in the absence of ATP and Mg^{2+}. Panels A, B and C correspond to 20, 30 and 40 nmol $Ca^{2+} \cdot mg^{-1}$, respectively. FCCP (0.5 μM) was added at the points shown by the arrows. RLM, rat liver mitochondria.

during its metabolism a transient Ca^{2+} release was observed, paralleled by an increase in the rate of oxygen consumption and mitochondrial swelling. The mitochondria, however, remained highly coupled, as shown in Fig. 2 (traces A, B and C) by the addition of FCCP. The experiments were performed varying the mitochondrial Ca^{2+} from 20 to 40 nmol $\cdot$ mg^{-1}. An increase in the hydroperoxide-induced effects was observed as the Ca^{2+} concentration increased. However, even at the highest Ca^{2+} load there was full re-uptake of the released Ca^{2+}, paralleled by the return of the respiration rate to the original values. The Ca^{2+} release and the increase in the rate of respiration were irreversible in the absence of ATP and Mg^{2+}, which showed to be extremely important in decreasing the rate and extent of mitochondrial Ca^{2+} release and swelling at all Ca^{2+} loads. A direct relationship between the rates of Ca^{2+} efflux and the extent of both Ca^{2+} load and hydrolysis of pyridine nucleotides was recently shown [46]. Therefore, the inhibitory effect of ATP observed under our conditions may suggest its regulatory role on the mitochondrial NADase and on the ability of Ca^{2+} retention by mitochondria. In addition, it

has been shown that both ATP and Mg^{2+} have an important stabilizing effect on the mitochondrial membrane [49,50].

Ca^{2+} flux during hydroperoxide metabolism in mitochondria energized by endogenous respiration plus exogenous ATP

Most of the experiments on the effect of NAD(P) redox state on mitochondrial Ca^{2+} efflux were performed in the presence of respiratory inhibitors and the reversibility of the pyridine nucleotides oxidation and Ca^{2+} release was shown by subsequent addition of NAD(P) reductants [22–36]. This has generated a controversy concerning the effect of redox states themselves on Ca^{2+} flux in mitochondria [22–27]. For instance, it was proposed that Ca^{2+} release induced by oxaloacetate was due to a direct effect of the oxidant on the mitochondrial membrane rather than to oxidation of NAD(P)H [22]. As a corollary, it was proposed that β-hydroxybutyrate reversed Ca^{2+} release by displacing oxaloacetate from its binding site on the membrane [22]. The experiment reported in Fig. 3 shows the reversibility of $NAD(P)^+$-stimulated Ca^{2+} efflux from mitochondria incubated in the absence of either inhibitors or reductants. Under these experimental conditions, reversible oxidation of NAD(P)H was caused

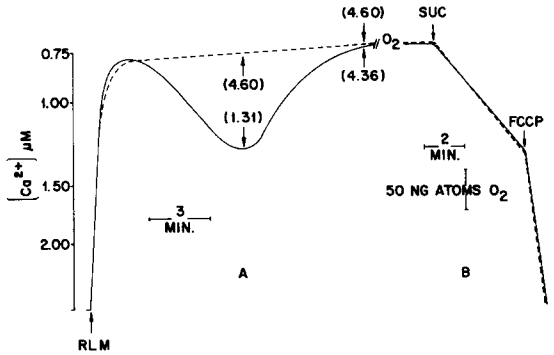

Fig. 3. Maintenance of respiratory control after a cycle of Ca^{2+} release and re-uptake induced by *t*-butylhydroperoxide in mitochondria energized by endogenous substrates plus ATP. Mitochondria (1.0 mg) were suspended in the basic medium (oligomycin, rotenone and succinate (suc), absent), in the presence (solid line) or absence (dashed line) of 40 μM *t*-butylhydroperoxide. Panel A shows the Ca^{2+} traces and panel B shows the rates of respiration after the addition of rotenone, oligomycin and succinate. FCCP (0.5 μm) was added where shown by the arrow. RLM, rat liver mitochondria.

by the addition of a limited amount of hydroperoxide which was paralleled by a transient Ca^{2+} release from mitochondria. When the Ca^{2+} steady state returned to the control levels, NADPH was found to be almost at the same concentration as in the control experiment. This result indicates that hydroperoxide-induced Ca^{2+} efflux from liver mitochondria is related directly or indirectly to the redox state of pyridine nucleotides.

After a cycle of Ca^{2+} release and re-uptake the degree of mitochondrial coupling was investigated. Thus, the addition of rotenone, oligomycin and succinate to these mitochondria led to the same rate of state-4 respiration compared to the control experiment. To prove that the state-4 respiration was actually a controlled state, FCCP was subsequently added. There was an immediate and very large acceleration of oxygen consumption, which was identical in both the hydroperoxide containing and control systems (Fig. 3B). The respiratory control ratio was about 7.5 in both cases.

Changes in membrane potential and mitochondrial swelling during the onset of Ca^{2+} efflux induced by hydroperoxide

The participation of $\Delta\psi$ on the mechanism of $NAD(P)^+$-stimulated Ca^{2+} efflux from mitochondria is controversial. Some workers claimed that $NAD(P)^+$-induced Ca^{2+} efflux is preceded by a collapse in $\Delta\psi$ [22,24–27], while others have shown that a decrease in $\Delta\psi$ during Ca^{2+} efflux is the result of a stimulated-energy-dissipating Ca^{2+} cycling across the mitochondrial membrane [30,31,51]. This question was studied under our experimental conditions where mitochondrial coupling was completely retained after the onset of Ca^{2+} release and re-uptake induced by hydroperoxide. It can be observed in Fig. 4 that the transient increase in extramitochondrial Ca^{2+} concentration is parallelled by a small decrease in $\Delta\psi$ (about 20 mV). The decrease in $\Delta\psi$ was smaller and the rate of net Ca^{2+} efflux was much faster when ruthenium red was added to the system containing hydroperoxide (dashed line). The rate of ruthenium red-induced Ca^{2+} efflux was 8 nmol $Ca^{2+} \cdot min^{-1} \cdot mg^{-1}$ in the presence of hydroperoxide whereas in its absence it was only 1 nmol $Ca^{2+} \cdot min^{-1} \cdot mg^{-1}$. The extent of mitochondrial swelling was also significantly decreased by

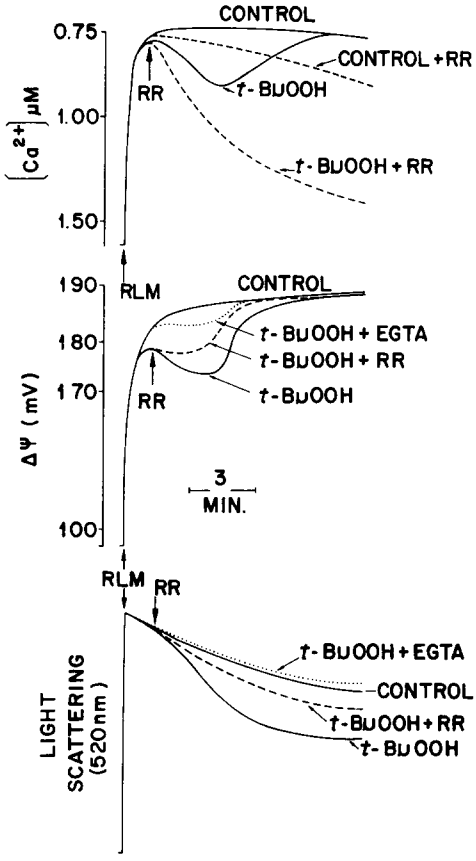

Fig. 4. Changes in $\Delta\psi$ and mitochondrial swelling during *t*-butylhydroperoxide induced Ca^{2+} release from liver mitochondria. Mitochondria (1.0 mg) were suspended in 1.0 ml of the basic medium in the presence or absence of 50 μM *t*-butylhydroperoxide. Ruthenium red (RR) (0.7 μM) was added where indicated (dashed lines). The dotted lines indicate an experiment carried out in the presence of 0.5 mM EGTA added prior to the addition of mitochondria. RLM, rat liver mitochondria.

ruthenium red. The addition of ruthenium red, which blocks Ca^{2+} cycling, was not followed by an immediate return of $\Delta\psi$ to the control values as it would be expected if the hydroperoxide-stimulated Ca^{2+} efflux was taking place through a $Ca^{2+}/2H^{+}$ antiporter. This also indicates that the observed decrease in $\Delta\psi$ cannot be attributed exclusively to an increased rate of Ca^{2+} cycling. Rather, it may be related to a transient increase in membrane permeability which would explain the parallel mitochondrial swelling. Addition of EGTA instead of ruthenium red caused similar alterations in both $\Delta\psi$ and swelling (not shown). The decrease in the extent of mitochondrial swelling upon the addition of ruthenium red is also in agreement with some previous reports [49,52,53] and corroborates the idea that mitochondrial swelling is increased by fast Ca^{2+} cycling [49,52, 53]. The dotted lines show the experiments where hydroperoxide was added to mitochondria suspended in a Ca^{2+}-free medium (EGTA present from the beginning). It can be observed that although the mitochondrial swelling was completely abolished in the absence of Ca^{2+} cycling there was still a small decrease in $\Delta\psi$. The nature of this decrease in $\Delta\psi$ is under study in this laboratory and appears to be related to the activity of the energy-linked transhydrogenase reaction using reducing equivalents from NADH to reduce $NADP^{+}$ and hydroperoxide driven by the electrochemical proton gradient ($\Delta\mu H^{+}$) (Vercesi, A.E., unpublished data).

It can be concluded that the decrease in $\Delta\psi$ observed in the presence of hydroperoxide is probably due to at least three different energy-dissipating processes: a faster Ca^{2+} cycling, the transhydrogenase reaction transferring reducing equivalents from NADH to $NADP^{+}$ and an increase in proton leak. The latter may explain the difference in $\Delta\psi$ observed between the conditions where EGTA was present from the beginning (dotted line) and where ruthenium red was added to prevent Ca^{2+} cycling (dashed line).

Correlation between the rate of O_2 consumption, NAD(P)H fluorescence and Ca^{2+} cycling induced by hydroperoxide

Fig. 5 shows a correlation between the time-course of the stimulation in O_2 consumption and the oxidation of NAD(P)H by hydroperoxide in Ca^{2+}-loaded mitochondria. If Ca^{2+} cycling was prevented by the addition of ruthenium red the hydroperoxide-induced increase in respiration was not completely abolished (dashed line) indicating that it was only partially caused by Ca^{2+} cycling. It can also be observed that even in a Ca^{2+}-free medium (EGTA present) hydroperoxide induced a small increase in the rate of respiration (dotted line). The different rates of O_2 consumption observed in this experiments (Fig. 5) reflect the different values of $\Delta\psi$ observed for the same experimental conditions in Fig. 4.

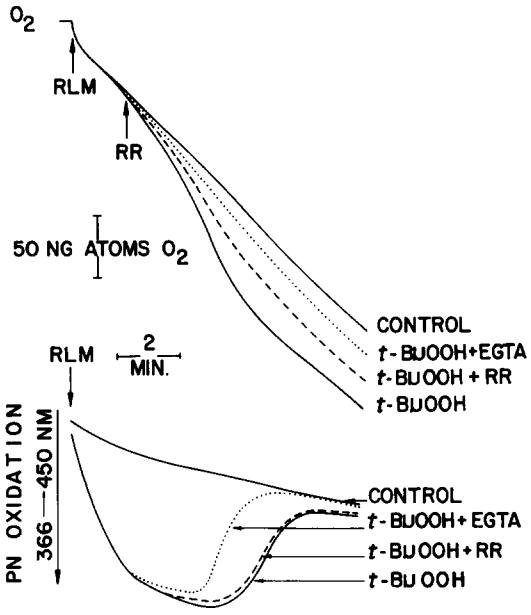

Fig. 5. Time-course of NAD(P)H oxidation and stimulation of O_2 consumption induced by *t*-butylhydroperoxide in liver mitochondria. Experimental conditions were exactly as in Fig. 4. RR, ruthenium red; RLM, rat liver mitochondria; PN, pyridine nucleotide.

The results of the fluorescence experiments showed that under these experimental conditions the re-reduction of pyridine nucleotides previously oxidized by hydroperoxide was reversible and occurred presumably at the expense of endogenous substrates. The earlier re-reduction observed in the presence of EGTA may reflect an inhibitory effect of Ca^{2+} on the transhydrogenase [54].

The results reported in the present paper indicate that under our experimental conditions the $NAD(P)^+$-induced transient increase in extramitochondrial Ca^{2+} concentration was independent of irreversible mitochondrial uncoupling and occurred in the absence of massive loss of internal pyridine nucleotides [30,31]. It was also recently shown by Frei et al. [46] that net loss of mitochondrial pyridine nucleotides during hydroperoxide-induced Ca^{2+} release was observed only at relatively high Ca^{2+} loads. These observations do not argue against the hypothesis that ADP-ribosilation of a protein in the inner mitochondrial membrane triggers Ca^{2+} release from liver mitochondria since it was shown that these mitochondria have the ability to rapidly resynthesize the hydrolysed nucleotides [46]. It should, however, be pointed out that this and a previous report from our laboratory [29] strongly indicate that in addition to the oxidation of NADH, NADPH must also be oxidized to stimulate Ca^{2+} release from mitochondria.

In conclusion, our results indicate that $NAD(P)^+$-stimulated Ca^{2+} efflux can be observed in tightly coupled mitochondria suspended in the presence of physiological concentrations of ATP and Mg^{2+}, but the ruthenium red insensitive decrease in $\Delta\psi$ observed during stimulated Ca^{2+} release and the parallel mitochondrial swelling do not support the idea that $NAD(P)^+$-induced Ca^{2+} release occurs through a $Ca^{2+}/2\ H^+$ antiporter.

Acknowledgements

This work was supported in part by grants of the Brazilian agencies FAPESP (Proc. 84/2483-4) and CNPq (Proc. 400239/84 BF).

References

1 Nicholls, D.G. (1978) Biochem. J. 176, 463–474
2 Becker, G.L. (1980) Biochim. Biophys. Acta 591, 234–239
3 Becker, G.L., Fiskum, G. and Lehninger, A.L. (1980) J. Biol. Chem. 255, 9009–9012
4 Pereira da Silva, L., Bernardes, C.F. and Vercesi, A.E. (1984) Biochem. Biophys. Res. Commun. 124, 80–86
5 Nichita, C.V. and Williamson, J.R. (1984) J. Biol. Chem. 259, 12978–12983
6 Lehninger, A.L. (1970) Biochem. J. 119, 129–138
7 Scarpa, A. and Azzone, G. (1970) Eur. J. Biochem. 12, 328–335
8 Selwin, M.J., Dawson, A.P. and Dunnet, S.J. (1970) FEBS Lett. 10, 1–5
9 Rottemberg, H. and Scarpa, A. (1974) Biochemistry 13, 1635–1646
10 Saris, N.E.L. and Åkerman, K.E.O. (1980) Curr. Topics Bioenerg. 10, 104–179
11 Nicholls, D. and Åkerman, K.E.O. (1982) Biochim. Biophys. Acta 683, 57–88
12 Åkerman, K.E.O. (1978) Biochim. Biophys. Acta 502, 359–366
13 Nicholls, D.C. (1978) Biochem. J. 170, 511–522
14 Fiskum, G. and Lehninger, A.L. (1979) J. Biol. Chem. 254, 6236–6239
15 Gunter, T.E., Chace, J.H., Puskin, J.I. and Gunter, K.K. (1983) Biochemistry 22, 6341–6351
16 Drahota, Z., Carafoli, E., Rossi, C.S., Gamble, P.L. and Lehninger, A.L. (1965) J. Biol. Chem. 240, 2712–2720
17 Moore, C.L. (1971) Biochim. Biophys. Res. Commun. 42, 298–305

18 Vasigton, F.D., Gazzotti, P., Tiozzo, R. and Carafoli, E. (1972) Biochim. Biophys. Acta 256, 43–54
19 Reed, K.C. and Bygrave, F.L. (1974) Biochem. J. 140, 143–155
20 Fiskum, G. and Lehninger, A.L. (1981) in Calcium and Cell Function (Cheung, W.Y., ed.), Vol. II, pp. 39–80, Academic Press, New York
21 Lehninger, A.L., Vercesi, A.E. and Bababunmi, E.A. (1978) Proc. Natl. Acad. Sci. USA 75, 1690–1694
22 Beatrice, M.C., Palmer, J.W. and Pfeiffer, D.R. (1980) J. Biol. Chem. 255, 8863–8671
23 Wolkowicz, P.E. and McMillin-Wood, J. (1980) J. Biol. Chem. 255, 10348–10353
24 Palmer, J.W. and Pfeiffer, D.R. (1981) J. Biol. Chem. 256, 6742–6750
25 Beatrice, M.C., Stiers, D.L. and Pfeiffer, D.R. (1982) J. Biol. Chem. 257, 7161–7171
26 Nicholls, D.G. and Brand, M.D. (1980) Biochem. J. 118, 113–118
27 Bardsley, M.E. and Brand, M.D. (1982) Biochem. J. 202, 197–201
28 Vercesi, A.E. and Lehninger, A.L. (1982) Fed. Proc. 41, 1434
29 Vercesi, A.E. (1986) Arch. Biochem. Biophys., in the press
30 Lötscher, H.R., Winterhalter, K.H., Carafoli, E. and Richter, C. (1979) Proc. Natl. Acad. Sci. USA 76, 4340–4344
31 Lötscher, H.R., Winterhalter, K.R., Carafoli, E. and Richter, C. (1980) J. Biol. Chem. 255, 9325–9330
32 Bellomo, G., Jewell, S.A., Thor, H. and Orrenius, S. (1982) Proc. Natl. Acad. Sci. USA 79, 6842–6846
33 Sies, H., Graf, P. and Estrela, J.M. (1981) Proc. Natl. Acad. Sci. USA 78, 3358–3362
34 Lötscher, H.R., Winterhalter, K.H., Carafoli, E. and Richter, C. (1979) Proc. Natl. Acad. Sci. USA 76, 4340–4344
35 Hofstetter, W., Mühlebach, T., Lötscher, H.R., Winterhalter, K.H. and Richter, C. (1981) Eur. J. Biochem. 117, 361–367
36 Richter, C., Winterhalter, K.H., Baumhuter, S., Lötscher, H.R. and Moser, B. (1983) Proc. Natl. Acad. Sci. USA 80, 3188–3192
37 Moser, B., Winterhalter, K.H. and Richter, C. (1983) Arch. Biochem. Biophys. 224, 358–364
38 Thor, H., Smith, M.T., Hartzell, P., Bellomo, G., Jewell, S.A. and Orrenius, S. (1982) J. Biol. Chem. 257, 12419–12425
39 Åkerman, K.E.O. and Nicholls, D.G. (1983) Rev. Physiol. Biochem. Pharmacol. 95, 149–201
40 Bernardes, C.F., Pereira da Silva, L. and Vercesi, A.E. (1985) 13th International Congress of Biochemistry, Amsterdam, The Netherlands, BBA Abstracts, p. 336, Elsevier Science Publishers B.V., Amsterdam
41 Kies, M.W. and Murphy, J.B. (1960) Biochim. Biophys. Acta 45, 382–384
42 Burgess, G.M., McKinneu, J.S., Fabiato, A., Leshie, B.A. and Putney, J.W., Jr. (1983) J. Biol. Chem. 258, 15336–15345
43 Beatrice, M.C., Stiers, D.L. and Pfeiffer, D.R. (1984) J. Biol. Chem. 259, 1279–1287
44 Kamo, N., Muratsugu, M., Hongoh, R. and Kobatake, J. (1979) J. Membrane Biol. 49, 105–121
45 Muratsugu, M., Kamo, N., Kurihara, K. and Kabatake, J. (1977) Biochim. Biophys. Acta 464, 613–619
46 Frei, B., Winterhalter, K.H. and Richter, C. (1985) Eur. J. Biochem. 149, 633–639
47 Bernardi, P., Paradisi, V., Pozzan, T. and Azzone, G.F. (1984) Biochemistry 23, 1645–1651
48 Zoccarato, F. and Nicholls, D.G. (1982) Eur. J. Biochem. 127, 333–338
49 Coelho, J.L.C. and Vercesi, A.E. (1980) Arch. Biochem. Biophys. 204, 141–147
50 Zoccarato, F., Rugolo, M., Silliprandi, D. and Siliprandi, N. (1985) Eur. J. Biochem. 114, 195–199
51 Baumhuter, S. and Richter, C. (1982) FEBS Lett. 148, 271–275
52 Moore, G.A., Jewell, S.A., Bellomo, G. and Orrenius, S. (1983) FEBS Lett. 153, 289–292
53 Bellomo, G., Martino, A., Richelmi, P., Moore, G.A., Jewell, S.A. and Orrenius, S. (1984) Eur. J. Biochem. 140, 1–6
54 Rydström, J. (1977) Biochim. Biophys. Acta 463, 155–184