Biochimica et Biophysica Acta, 936 (1988) 139–147
Elsevier

BBA 42859

Mechanism of nitrofurantoin toxicity and oxidative stress in mitochondria

Donatella Carbonera, Alessandro Angrilli and Giovanni Felice Azzone

C.N.R. Unit for Physiology of Mitochondria and Institute of General Pathology, University of Padova, Padova (Italy)

(Received 20 April 1988)

Key words: Nitrofurantoin toxicity; Oxidative stress; (Mitochondria)

5-Nitrofuran derivatives change the inner mitochondrial membrane permeability as indicated by the transmembrane potential, the rate of spontaneous K^+ efflux and the basal respiratory rate: (a) at low concentrations nitrofurantoin prevents the increase of inner membrane permeability due to hydroperoxides or to diamide; (b) at higher concentrations or after longer times of incubation, nitrofurantoin enhances the membrane damage due to hydroperoxides or to diamide; the damage due Ca^{2+} plus P_i is enhanced by nitrofurantoin at all concentrations; (c) higher nitrofurantoin concentrations cause membrane damage independently of the presence of hydroperoxides or of diamide. The effect of nitrofurantoin is cancelled by the addition of free-radical scavengers. The above effects of nitrofurantoin are compatible with the observations of Mason and collegues that nitrofurantoin is reduced by a NADPH nitroreductase to a nitro anion radical which can then undergo subsequent reactions, among which are (a) initiation of a free-radical reaction chain and (b) reduction of hydroperoxides and diamide.

Introduction

5-Nitrofuran derivatives have been used in mitochondrial research for quite different purposes. They are very useful antibacterial agents, although under some conditions they also have toxic or mutagenic effects [1,2]. It was suggested that these toxic effects are, at least in part, related to the formation of activated forms of oxygen as indicated by the potentiating effects of deficiencies of vitamin E [3] and of selenium [4]. Pharmacological concentrations of nifurtimox induce maximal stimulation of superoxide anion production by *Trypanosoma cruzi* and initiate H_2O_2 diffusion outside the cell [5,6]. Mason et al. [7,8] showed that nitrofurantoin is enzymatically converted to nitro anion radicals capable of participating in redox cycling in mitochondria.

Nitrofurantoin has been used to support the concept that the permeability of the mitochondrial inner membrane is controlled by the GSSG/GSH ratio, which in turn controls the state of reduction of critical SH groups [9]. These critical SH groups regulate the activity of the SH-dependent lysophospholipid acyltransferase activity and consequently the ability of mitochondria to remain impermeable upon activation of the intramitochondrial phospholipase A_2 by Ca^{2+}. The increased inner membrane permeability produced by nitrofurantoin was related to its capacity to inhibit the glutathione reductase activity with depletion of GSH. Metabolism of hydroperoxides results in formation of GSSG and consumption

Abbreviations: BuOOH, butylhydroperoxide; BHT, butylhydroxytoluene; Diamide, azodicarboxylic acid bis(dimethylamide) or diazinecarboxylic acid bis(*N*,*N*-dimethylamide); Nifurtimox, 4-(5-nitrofurfurylideneamino)-3-methylethiomorpholine 1,1,-dioxide; BSA, bovine serum albumin.

Correspondence G.F. Azzone, Istituto di Patologia Generale, Via Loredan, 16, 35131 Padova, Italy.

NADPH and causes Ca^{2+} efflux from the hepatocyte [10]. Nitrofurantoin also perturbs liver redox metabolism, increases efflux of GSSG into the bile [11].

There is increasing evidence that mitochondrial damage, as well as cell damage, is due to oxidative stress [12–18]. The mitochondrial damage induced by Ca^{2+} is markedly enhanced by the addition of hydroperoxides [19] and diamide [20], and when isolated liver mitochondria are treated with hydroperoxides, free radicals are detected by a spin-trapping technique [21]. We have reported [22,23] that several Ca^{2+}-dependent increases in inner membrane permeability [24–26] presumably involve the formation of free radicals and are abolished by antioxidants. In the present investigation we determined the effect of nitrofurantoin on the increase of inner membrane permeability. Contrary to expectations, addition of low nitrofurantoin concentrations does not enhance, but rather inhibits, the increase of inner membrane permeability caused by Ca^{2+}- and peroxides or diamide. On the other hand, the inner membrane damage is enhanced at higher nitrofurantoin concentrations or after longer times of incubation, or when the damage is due to the presence of Ca^{2+} plus phosphate. Under all these conditions, the nitrofurantoin-induced enhancement of inner membrane damage is abolished by antioxidants. The implications of these observations are discussed.

Experimental

Rat liver mitochondria were prepared in 0.25 M sucrose/10 mM Tris-HCl (pH 7.4)/0.1 mM EGTA [27]. The last washing was carried out in an EGTA-free medium and the final resuspension made in 0.1% BSA-supplemented medium. Mitochondrial protein was assayed with the biuret method with BSA as standard.

The composition of the incubation media is specified in the legends.

The transmembrane potential was determined by monitoring the Ph_3MeP^+ concentration in the bulk phase with a Ph_3MeP^+-sensitive electrode. Medium Ph_3MeP^+ concentrations were calculated from the continuous plot of the electrode output vs. the Ph_3MeP^+ concentrations as determined for each individual experiment.

The rate of oxygen uptake was followed polarographically with a Clark oxygen electrode. Oxygen concentration was taken as 485 natoms oxygen/ml at 25 °C.

K^+ effluxes were monitored with a K^+-sensitive electrode at 25 °C. Calibration of the electrode was performed by multiple additions of known amounts of KCl before each experiment.

Hydroperoxides and butylhydroxytoluene were kindly provided by Prof. F. Ursini. All other chemicals were commercial products of the highest purity.

Results

The effect of nitrofurantoin on transmembrane potential

Addition of 30 μM hydroperoxides to mitochondria supplemented with 30 μM Ca^{2+} resulted in the usual biphasic effect of potential decline: a rapid phase, presumably linked to the initiation of the transhydrogenase reaction, and a second, slower phase in parallel with the damage of the mitochondrial inner membrane (Fig. 1A). When the hydroperoxides were added to mitochondria already supplemented with 100 μM nitrofurantoin both the rapid and the slow phase were abolished. The abolition of the rapid phase is due to the inhibition by nitrofurantoin of the glutathione reductase reaction [9], resulting in a diminished utilization of NADPH and activity of the transhydrogenase reaction. Nitrofurantoin also abolished the slower phase of $\Delta\psi$ depression attributed to the formation of oxygen radicals [22,23]. When nitrofurantoin was added after the hydroperoxides, the inhibitory effect was evident only in the slow phase. Fig. 1B shows the effect of increasing concentrations of nitrofurantoin on the decline of the transmembrane potential caused by the hydroperoxides. In the absence of nitrofurantoin, addition of 50 μM hydroperoxides caused a cycle of potential decline which eventually levelled off and then was followed by a partial restoration of the potential. In the presence of increasing concentrations of nitrofurantoin a dual effect was apparent: there was initially a partial abolition both of the rapid and of the slow phase of poten-

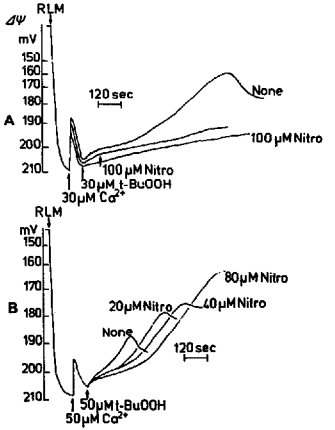

Fig. 1. Protection by nitrofurantoin (Nitro) of hydroperoxide-induced $\Delta\psi$ decline in mitochondria. The incubation medium contained 200 mM sucrose; 10 mM Tris-Mops (pH = 7.4), 5 mM succinate-Tris, 2 μM rotenone, 0.5 mM P_i, 1 mg/ml bovine serum albumin, 1 mg/ml mitochondria and 5 μM Ph_3MeP^+ at 25° C. The traces show the pattern of $\Delta\psi$. Ca^{2+}, tBuOOH and nitrofurantoin were added at the incubated concentrations.

tial decline, while in the long term the pattern was changed in that the final potential was lower in the presence than in the absence of nitrofurantoin. Thus, at longer incubation times there was an enhancement of the damage of the inner membrane with increasing nitrofurantoin concentrations.

Fig. 2A shows the results of replacing hydroperoxides with diamide. Nitrofurantoin had a dual effect similar to those caused by hydroperoxides. At shorter incubation times, the presence of nitrofurantoin resulted in a partial abolition of the decline in potential, while at longer incubation times there was an enhanced damage of the membrane as indicated by a more marked decline in potential. Fig. 2B shows the effect of increasing nitrofurantoin concentration on the inner membrane damage caused by Ca^{2+} plus P_i was not diminished by nitrofurantoin and the increased damage was proportional to the nitrofurantoin concentration.

The effect of nitrofurantoin on K^+ release and respiratory rate

Fig. 3 shows the effect of hydroperoxides and of nitrofurantoin on K^+ release. Addition of 100 μM hydroperoxides initiated a permeability increase which led to a K^+ release reaching completion in about 2 min. In the presence of 5 μM nitrofurantoin the process of K^+ release was delayed and decelerated. However complete release of matrix K^+ was still obtained in about 4 min. At higher concentrations of nitrofurantoin, on the other hand, there was complete abolition of K^+ release, indicating that nitrofurantoin is capable of abolishing the damage to the inner membrane caused by the hydroperoxides. The efflux of K^+ in Fig. 3 indicates that, in the presence of Ca^{2+} and hydroperoxides, the membrane has increased its permeability not only to K^+ but also to some other ion species neutralizing the K^+ flux. This ion species is presumably H^+, as indicated by the

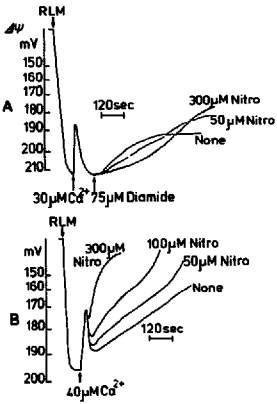

Fig. 2. Effect of increasing nitrofurantoin concentration on diamide- (A) and Ca^{2+} plus P_i (B) induced $\Delta\psi$ decline. The incubation medium was as in Fig. 1. Ca^{2+}, diamide and nitrofurantoin were added to the medium at the indicated concentrations. In (B) P_i was 3 mM.

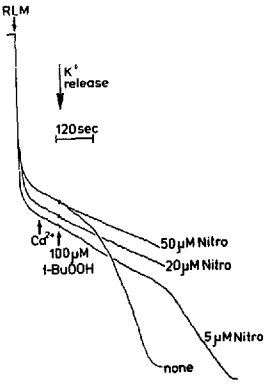

Fig. 3. Effect of nitrofurantoin on tBuOOH-induced K^+ efflux. The incubation medium was as in Fig. 1 in absence of BSA. The traces indicate the K^+ fluxes at increasing nitrofurantoin concentrations. When indicated 60 µM Ca^{2+}, 100 µM tBuOOH.

parallel decline of the membrane potential.

The effect of nitrofurantoin on the kinetics of the respiratory stimulation caused by hydroperoxides and diamide was also studied. Similarly to the case of the decline in potential, the respiratory stimulation was a gradually increasing process reaching a maximum on a time-scale of several minutes. Addition of nitrofurantoin was able to delay and to depress the respiratory stimulation caused by hydroperoxides or by diamide.

Fig. 4 shows the effect of increasing nitrofurantoin concentrations on the respiratory stimulation caused by hydroperoxides. The respiratory rate increased from about 30 natoms · mg^{-1} · min^{-1} at 2 min to about 150 natoms · mg^{-1} · min^{-1} at 7 min after the addition of the hydroperoxides. However, the entire respiratory stimulation caused by the hydroperoxides was abolished by nitrofurantoin. Increase of the nitrofurantoin concentration from 50 to 300 µM did not change the pattern in the hydroperoxide-supplemented mitochondria, at variance with the situation in diamide-supplemented mitochondria, cf. Fig. 5.

Fig. 5 shows the effect of increasing nitrofuran-

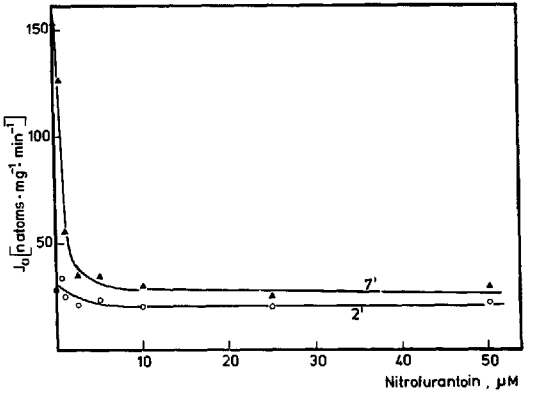

Fig. 4. Effect of increasing nitrofurantoin concentrations on the hydroperoxide-induced stimulation of the respiratory rate. The incubation medium was as in Fig. 1. The mitochondria were supplemented with 50 µM Ca^{2+} and 150 µM peroxide. The values of the respiratory rate are those taken at 2 and 7 min after tBuOOH addition.

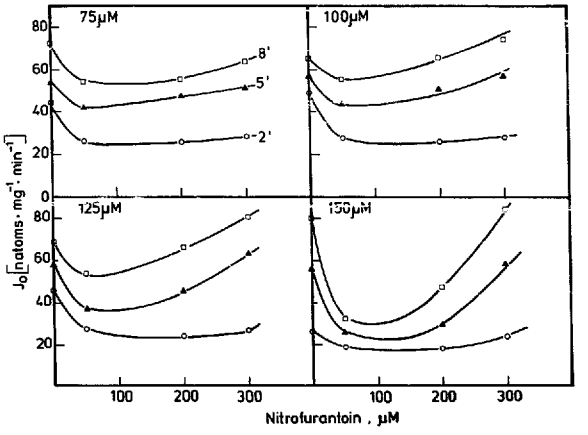

Fig. 5. Effect of increasing nitrofurantoin concentrations on the diamide-induced stimulation of the respiratory rate. The incubation medium was as in Fig. 1. The mitochondria were supplemented with 30 μM Ca^{2+}. The concentrations of diamide where indicated. The values of the respiratory rate are shown at 2, 5, 8 min after diamide addition.

toin concentrations at various times of incubation on the respiratory stimulation caused by increasing diamide concentrations. As in the case of the hydroperoxides, almost the entire respiratory stimulation was abolished by nitrofurantoin when added at low concentrations, say below 50 μM. On the other hand, in the diamide-supplemented mitochondria, when nitrofurantoin was added at higher concentration the abolition of the respiratory stimulation was replaced by an enhancement of the respiration. Furthermore, the respiratory rate increased with increased incubation time. These effects agree with the enhancement of the decline in membrane potential (Fig. 2).

Fig. 6 shows the effect of increasing nitrofurantoin concentrations on the respiratory stimulation caused by Ca^{2+} plus P_i. The addition of nitrofurantoin had no protective effect, but rather enhanced the respiratory stimulation. This agrees with the enhancement of the decline in membrane potential caused by Ca^{2+} plus P_i (Fig. 2). These observations suggest that fundamentally different processes are initiated by hydroperoxides and diamide, and by and Ca^{2+} plus P_i.

Fig. 7 shows the effect of increasing butylhydroxytoluene concentrations on the respiratory stimulation caused by 300 μM nitrofurantoin. It is seen that the respiratory stimulation was very sensitive to the presence of butylhydroxyltoluene, 50% inhibition being obtained at about 10 μM butylhydroxytoluene. The results of Fig. 7 suggest that the mechanism of the respiratory stimulation following the addition of nitrofurantoin to rat liver mitochondria involves the formation of oxygen radicals. Mitochondria were supplemented also with 150 μM diamide and 300 μM nitrofurantoin, concentrations which were found in the experiment of Fig. 5, to enhance the respiratory stimulation caused by diamide particularly at longer times of incubation. Also this stimulatory effect of nitrofurantoin was sensitive to the addition of antioxidants, although somewhat higher butylhydroxytoluene concentrations were required for protection.

Discussion

In the present study we report three effects. First nitrofurantoin at low concentrations or at

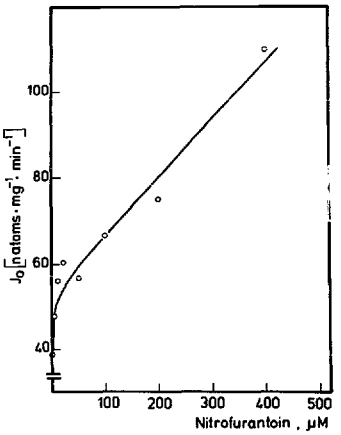

Fig. 6. Effect of increasing nitrofurantoin concentrations on the Ca^{++} plus P_i-induced stimulation of the respiratory rate. The incubation was as in Fig. 1 except that P_i was 3 mM. The mitochondria were supplemented with 70 µM Ca^{2+}.

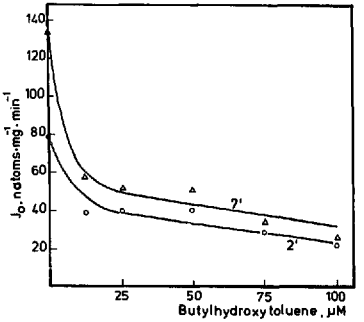

Fig. 7. Effect of increasing butylhydroxytoluene concentrations on the nitrofurantoin-induced stimulation of the respiratory rate. The medium was as in Fig. 1. 300 µM nitrofurantoin, 0.5 mM P_i. The respiratory rates are shown 2 and 7 min after nitrofurantoin addition.

short times of incubation abolishes the increase of inner membrane permeability caused by hydroperoxides and diamide. Second, nitrofurantoin at higher concentrations or at longer times of incubation enhances the increase of inner membrane permeability by hydroperoxides or diamide. Furthermore, nitrofurantoin at all concentrations or times of incubation causes an increase of inner membrane permeability caused by Ca^{2+} plus P_i. Third, nitrofurantoin alone increases the permeability of the inner membrane in the absence of hydroperoxides, diamide or of Ca^{2+} plus P_i. The questions arise: (a) whether the different effects are the expression of a multiplicity or of a single mode of action of nitrofurantoin; and (b) whether the different actions are compatible only with inhibition of glutathione reductase by nitrofurantoin.

Pfeiffer [9] has suggested that there is a correlation between level of GSH and permeability of the inner mitochondrial membrane. Subsequent work [22] has, however, shown that: (a) the damage induced by Ca^{2+} plus P_i is not accompanied by decrease in GSH levels and (b) that the protection by free-radical scavengers with respect to the damage due to hydroperoxides is not accompanied by restoration or maintenance of a high level of GSH. The finding of the present study that low concentrations of nitrofurantoin abolish rather than enhance the damage caused by hydroperoxides and diamide is also in contrast with the view that the effects of nitrofurantoin can be explained exclusively on the basis of its inhibitory action on the glutathione reductase. In fact, such an inhibition by nitrofurantoin would cause a further decrease in the level of GSH in the presence of hydroperoxides and diamide and thus increase rather than decrease the damage.

Several lines of evidence, on the other hand, suggest that free radicals play an important role in determining this multiplicity of effects caused by nitrofurantoin. Firstly, the protection obtained by radical scavengers indicates that the inner membrane damage due to hydroperoxides, diamide or Ca^{2+} plus P_i is the consequence of an oxidative stress [22,23]. Secondly, Mason et al. [7,8] have shown that nitrofurantoin is reduced in rat liver mitochondria, by means of an NADPH nitroreductase localized on the outer membrane, to nitro

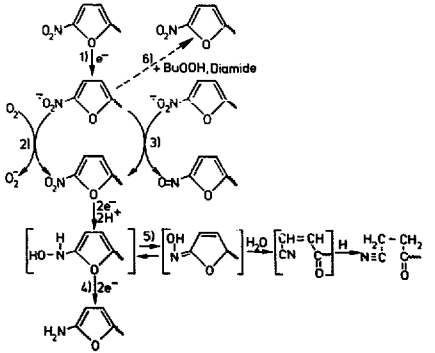

Fig. 8. Possible reactions of nitrofurantoin in mitochondria.

anion radicals whose oxidation should regenerate the superoxide anion catalytically. The lack of effect of superoxide dismutase and catalase on the steady-state concentration of the nitro anion radical was taken as evidence that neither superoxide anion nor hydrogen peroxide is an intermediate in the generation of the anion radical. Thirdly, the effect of increased membrane damage by nitrofurantoin is also removed by free-radical scavengers. Mason et al. [7,8] observed a stimulation of the oxygen consumption after addition of nitrofurantoin to rat liver mitochondria but attributed the stimulation only to redox cycling of the nitro anion radicals with generation of superoxides.

For the purpose of the present study, it is immaterial whether the nitroreductase reaction occurs mainly on the outer mitochondrial membrane as indicated by Mason and co-workers [7,8], or partially on the inner membrane also, as suggested by the insensitivity of the nitro anion radical concentration to superoxide dismutase or catalase. We consider that the insensitivity of the nitrofurantoin-caused membrane damage to superoxide dismutase or catalase cannot distinguish between absence of an intermediate or lack of accessibility of the intermediate to the enzyme. Our results are thus essentially in agreement with the observations of Mason and co-workers [7,8] but indicate that the formation of the nitro anion radicals by means of the nitroreductase reactions has further consequences for the properties of the mitochondria.

Fig. 8 presents a scheme summarizing a number of possible reactions of nitrofurantoin in mitochondria. Reaction 1 is a nitroreductase reaction [7,8] or any other equivalent reaction leading to the formation of nitro anion radicals. The nitro anion radical can undergo several types of reaction. Reaction 2, interaction of the nitro anion radical with oxygen leads to the formation of the superoxide anion which can then initiate a free-radical reaction chain. Reaction 3, a dismutation reaction between two nitro anion radicals leads to regeneration of the nitrofurantoin molecule and to formation of a nitroso compound. Reactions 4 and 5 are alternative pathways of reaction of nitrofurantoin leading to the formation of hydroxyl intermediates, to amines and to nitriles, respectively. Of particular importance is reaction 6, where the nitro anion radical reduces the hydroperoxides or diamide with removal of hydroperoxides and of diamide from the medium; since the reduction of the hydroperoxides and of diamide would result in regeneration of the nitrofurantoin molecule, reaction 6 would explain how a catalytic

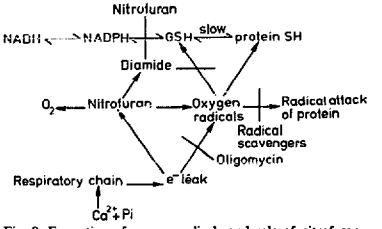

Fig. 9. Formation of oxygen radicals and role of nitrofurantoin.

amount of nitrofurantoin could cancel the effects of much larger concentrations of hydroperoxides and diamide. In conclusion, the reaction shown in the scheme of Fig. 8 explain all the effects of nitrofurantoin observed in the present study, namely: (a) the abolition by low nitrofurantoin concentrations of the damaging effects of hydroperoxides and diamide (reaction 6); (b) the enhancement by higher nitrofurantoin concentrations, or at longer times of incubation, of the damage caused by hydroperoxides and diamide (reaction 2); (c) the damage caused by high nitrofurantoin concentrations even in the absence of hydroperoxides and diamide (again reaction 2) – the predominance of reaction 2 would also explain the damage observed at all nitrofurantoin concentrations in the presence of Ca^{2+} plus P_i; and (d) the relationship between protective nitrofurantoin concentrations and damaging diamide concentrations (competition between reactions 3, 4 and 5 on the one side and 6 on the other).

Fig. 9 shows another scheme summarizing both present and previous information [22] with respect to the damage caused by oxygen radicals in mitochondria. It is assumed that an electron leak from the respiratory chain leads to a univalent oxygen reduction with formation of oxygen radicals which initiate the radical attack of proteins and lipids. The radical attack is prevented by the addition of radical scavengers and potentiated by the addition of agents, such as the hydroperoxides or diamide, which presumably either directly or indirectly, for example via a decreased concentration of GSH, lead to a higher concentration of radicals. Nitrofurantoin gives rise to nitro anion radicals which undergo alternative reactions as illustrated in the scheme of Fig. 8. The possibility is not excluded that part of the effect of nitrofurantoin might be also due to its inhibitory action of the glutathione reductase reductase reaction. In fact, such an inhibition would result in a decreased concentration of GSH and then in a decreased removal of oxygen radicals. However, the sensitivity to the free-radical scavengers and independence of the level of GSH presumably render this glutathione reductase inhibition less relevant [22].

Two comments are in order with respect to the damage by Ca^{2+} plus P_i and to the involvement of Ca^{2+} in the radical damage. The permeability increase caused by Ca^{2+} plus P_i was insensitive to the protective effect of nitrofurantoin. Presumably the nitro anion radical cannot interfere with the pathway of membrane damage operating in the presence of Ca^{2+} plus P_i. On the other hand, it is highly significant that all butylhydroxytoluene-sensitive processes leading to damage of the inner membrane, show also a requirement for Ca^{2+}. It may be conceived that the Ca^{2+} requirement reflects the need to loosen the membrane structure in order to enhance the electron leak from the respiratory chain, thus leading to the formation of the oxygen radicals. The activation of Ca^{2+}-dependent intramitochondrial phospholipases as proposed by Pfeiffer et al. [28,29,31] and by Siliprandi et al. [30], may perhaps be the means by which the free-radical damage becomes dependent on the matrix accumulation of Ca^{2+}. It is interesting that the Ca^{2+}-induced potentiation of oxygen free-radical injury in renal mitochondria is due in part to activation of phospholipase A_2 as indicated by the protective effect of dibucaine [32]. The Ca^{2+}-dependent oxygen radical attack on the inner membrane may eventually lead to the formation of pores, as suggested by Crompton et al. [33,34].

References

1 Docampo, D.R. and Moreno, S.N.J. (1984) in Free Radicals in Biology (Pryor W.A., ed.), Vol. 6, pp. 243–288, Academic Press, New York.

2 Cohen, S.M. (1978) in Carcinogenesis -A Comprehensive Study (Bryan, G.T., ed.), Vol. 4, pp. 171–231, Raven Press, New York.
3 Boyd, M.R., Catignani, G.L., Sasame, H.A., Mitchell, J.R. and Stiko, A.W. (1979) Am. Rev. Respir. Dis. 120, 93–99.
4 Peterson, F.J., Combs, G.F., Jr., Holtzmann, J.L. and Mason, R.P. (1982) J. Nutr. 112, 1741–1746.
5 Docampo, R., and Stoppani, A.O.M. (1979) Arch. Biochem. Biophys. 197, 317–321.
6 Docampo, R., Moreno, S.N.J., Stoppani, A.O.M., Leon, W., Cruz, F.S., Villalta, F. and Muniz, R.P.A. (1981) Biochem. Pharmacol. 30, 1947–1951.
7 Docampo, R. Mason, R.P. Mottley, C. and Muniz, R.P.A. (1981) J. Biol. Chem. 256, 10930–10933.
8 Moreno, S.N.J. Mason, R.P. and Docmpo, R. (1984) J. Biol. Chem. 259, 6298–6305.
9 Beatrice, M.C., Stiers, D.L. and Pfeiffer, D.R. (1984) J. Biol. Chem. 259, 1279–1287.
10 Bellomo, G., Jewell, S.A., Thor, H. and Orrhenius, S. (1982) Proc. Natl. Acad. Sci. USA 79, 6842–6846.
11 Dubin, M., Moreno, S.N.J., Martino, E.E., Docampo, R. and Stoppani, A.O.M. (1983) Biochem. Pharmacol. 32, 483–487.
12 Hunter, F.E., Gebicki, J.M., Hoffsten, P.E., Winstein, J. and Scott A. (1963) J. Biol. Chem. 238, 828–835.
13 Hunter, F.E., Scott, A., Hoffsten, P.E., Gebicki, J.M., Weinstein, J. and Schneider, A. (1964) J. Biol. Chem. 239, 614–621.
14 Thor, H., Smith, M.T., Hartzell, P. Bellomo, G. Jewell, S.A. and Orrhenius, S. (1982) J. Biol. Chem. 257, 12419–12425.
15 Novgorod, S.A., Kultayeva, E.V. Yaguzhinsky, L.S. and Lemesshko V.V. (1987) J. Bioener. Biomem. 19, 191–202.
16 Aust, S.D., Morehouse, L.A. and Craig, C.E. (1985) J. Free Rad. Biol. Med. 1, 3–25.
17 Wolff, S.P., Garner, A. and Dean, R.T. (1986) Trends Biochem. Sci. 11, 27–31.
18 Pascoe, G.A., Fariss, M.W., Olafsdottir, K. and Reed, D.J. (1987) Eur. J. Biochem. 166, 241–247.
19 Beatrice, M.C., Stiers, D.L. and Pfeiffer, D.R. (1982) J. Biol. Chem. 257, 7161–7171.
20 Rugolo, M., Siliprandi, D., Siliprandi, N. and Toninello, A. (1981) Biochem. J. 200, 481–486.
21 Kennedy, C.H., Pryor, W.A., Winston, G.W. and Church, D.F. (1986) Biochem. Biophys. Res. Commun. 141, 1123–1129.
22 Carbonera, D. and Azzone, G.F. (1988) Biochim. Biophys. Acta, in press.
23 Carbonera, D., Angrilli, A. and Azzone, G.F. (1983) 5th European Bioenergetics Conference, Aberystwith, p. 126, Elsevier, Cambridge.
24 Chappel, J.B. and Greville, G.D. (1963) Biochem. Soc. Symp. 23, 23–39.
25 Azzi, A. and Azzone, G.F. (1965) Biochim. Biophys. Acta 105, 253–264.
26 Azzi, A. and Azzone, G.F. (1966) Biochim. Biophys. Acta 113, 438–464.
27 Massari, S. Balboni, E. and Azzone, G.F. (1973) Biochim. Biophys. Acta 283, 16–22.
28 Pfeiffer, D.R., Kaufman, R.F. and Lardy, H.A. (1978) J. Biol. Chem. 253, 4165–4171.
29 Pfeiffer, D.R., Schmid, P.C., Beatrice, M.C. and Schmid, H. (1979) J. Biol. Chem. 254, 11485–11494.
30 Siliprandi, D., Rugolo, M., Zoccarato, F., Toninello, A., and Siliprandi, N. (1979) Biochem. Biophys. Res. Commun. 88, 388–394.
31 Beatrice, M.C., Palmer, J.W. and Pfeiffer, D.R. (1980) J. Biol. Chem. 255, 8663–8671.
32 Malis, C.D. and Bonventre, J.V. (1986) J. Biol. Chem. 261, 14201–14208.
33 Ai Nasser and Crompton, M. (1986) Biochem. J. 239, 18–29.
34 Crompton, M., Costi, A. and Hayat, L. (1987) Biochem. J. 245, 915–918.