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Damage to rat liver mitochondria promoted by δ-aminolevulinic acid-generated reactive oxygen species: connections with acute intermittent porphyria and lead-poisoning

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δ-Aminolevulinic acid is a heme precursor accumulated in acute intermittent porphyria and lead-poisoning, which supposedly triggers the typical clinical expression associated with these diseases. Considering that: (i) erythrocyte anti-oxidant enzymes are abnormally high in patients with both disorders and (ii) δ-aminolevulinic acid autoxidation generates reactive oxygen species, a possible contribution of reactive oxygen species in the pathophysiology of these disorders is explored here. Evidence is provided that δ-aminolevulinic acid (2–15 mM) induces damage to isolated rat liver mitochondria. Addition of δ-aminolevulinic acid disrupts the mitochondrial membrane potential, promotes Ca^{2+} release from the intramitochondrial matrix and releases the state-4 respiration, thus enhancing the permeability of the membrane to H^+. The lesion was abolished by catalase, superoxide dismutase (both enzymes inhibit δ-aminolevulinic acid autoxidation) and *ortho*-phenanthroline, but not by mannitol; added H_2O_2 induces damage poorly. These results suggest the involvement of deleterious reactive oxygen species formed at particular mitochondrial sites from transition metal ions and δ-aminolevulinic acid-generated peroxide and/or superoxide species. These observations might be compatible with previous work showing hepatic mitochondrial damage in liver biopsy samples of acute intermittent porphyria patients.

Introduction

Acute intermittent porphyria and lead poisoning are diseases in which acute attacks of abdominal pain and neuropsychiatric manifestations have been linked to accumulation of δ-aminolevulinic acid – a precursor of heme biosynthesis [1–3]. δ-Aminolevulinic acid accumulates as a result of abnormally low biosynthesis of uroporphyrinogen I synthetase in acute intermittent porphyria and of lead inhibition of δ-aminolevulinic acid dehydrase and ferrochelatase in lead poisoning [1–3]. Liver- and bone-marrow-generated δ-aminolevulinic acid is able to cross the blood-brain barrier and be distributed in the central nervous system and in other organs [4,5].

The biochemical basis whereby δ-aminolevulinic acid accumulation leads to toxicity remains unclear [2,3]. At the neurochemical level, 10^{-6} M δ-aminolevulinic acid inhibits the K^+ -stimulated release of the neurotransmitter γ-aminobutyric acid from preloaded synaptosomes [6] and also inhibits the binding of γ-aminobutyric acid to synaptic membranes [7]. However, these observations do not explain the neuropathological changes, such as axonal degeneration and demyelinization, that occur in acute intermitent porphyria and lead poisoning [1,2,8]. In addition, hepatic damage, including ultrastructural alterations in endoplasmic reticulum and mitochondria, is observed in acute intermitent porphyria [9]. Moreover, impairement of kidney mitochondria function has been reported in lead poisoning [1]. The origin of these dysfunctions is still obscure.

Abbreviations: RLM, rat liver mitochondria; $\Delta\Psi$, mitochondrial membrane potential; TPP^+, tetraphenyl-phosphonium bromide; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

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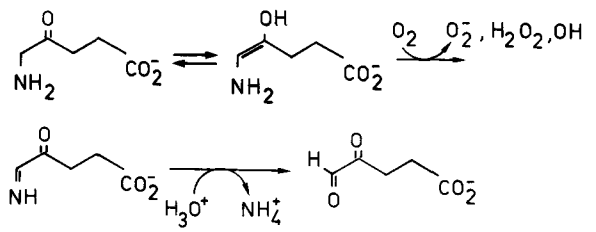

Scheme I.

We [10–14] have presented an alternative rationale concerning the biochemical bases of these disorders, by connecting δ-aminolevulinic acid accumulation to oxidative stress. The evidence for this is as follows: (i) enolic δ-aminolevulinic acid forms reactive oxygen species (O_2^-, H_2O_2, $OH^\cdot$) by autoxidation (Scheme I), concomitant to oxidation of oxyhemoglobin to methemoglobin [10–12] and (ii) erythrocyte levels of superoxide dismutase and of glutathione peroxidase are enhanced in acute intermittent porphyria patients [13] and in lead-exposed workers from industries of the State of São Paulo (Brazil) [14]. These results, taken as a whole, were interpreted in terms of δ-aminolevulinic acid-generated reactive oxygen species as the etiological agents in acute intermittent porphyria and lead poisoning [10–14].

Here, we present evidence of damage for isolated rat liver mitochondria (RLM) by δ-aminolevulinic-acid-generated reactive oxygen species as a model to explain the pathophysiology of acute intermittent porphyria and lead poisoning at the cellular level. Mitochondrial membrane potential ($\Delta\Psi$), Ca^{2+} fluxes and respiratory rate were monitored both in the presence and absence of δ-aminolevulinic acid. The effects of catalase, superoxide dismutase, mannitol, *ortho*-phenanthroline (*o*-phen) and H_2O_2 on δ-aminolevulinic acid-induced lesion to mitochondria were also studied.

Materials and Methods

Reagents. δ-Aminolevulinic acid hydrochloride, tetraphenylphosphonium bromide (TPP^+), bovine liver catalase, bovine blood superoxide dismutase, horseradish peroxidase type VI and (Hepes) were purchased from Sigma Chemical Co. *ortho*-Phenanthroline was from Merck. Stock solutions of δ-aminolevulinic acid hydrochloride (250 mM) were stored at −20 °C. All other reagents were of analytical grade.

Preparation of δ-aminolevulinic-acid-containing media. After addition of δ-aminolevulinic acid to the incubation media, the pH was adjusted to 7.2 with Tris, and the incubated 15 min at room temperature (25 °C). The experiments were started by the addition of succinate or mitochondrial suspension (see legend to figures).

Isolation of RLM. Rat liver mitochondria were isolated as previosly described [15] from overnight-fasted male Wistar rats weighing about 250 g.

Measurement of $\Delta\Psi$. Mitochondria were incubated in the reaction medium containing 3 μM TPP^+ as a probe. The changes in TPP^+ activity in the extramitochondrial medium (due to its migration to the internal mitochondrial compartment) was continuously monitored with a TPP^+-selective electrode prepared in our laboratory according to Kamo et al. [16]. The electrode signals were amplified and the output registered with a dual-channel recorder (Linear Model 1202). The mitochondrial membrane potential was calculated assuming that the TPP^+ distribution between mitochondria and medium follows the Nernst equation [17].

Measurement of Ca^{2+} fluxes and oxygen uptake. Changes in Ca^{2+} concentration in the incubation media (10 μM, measured by atomic absorption) were followed using a Ca^{2+}-selective electrode (Radiometer F21112 Calcium Selectrode). Signals from the Ca^{2+} electrode were fed into an amplifier and then into a dual-channel recorder (Linear Model 1202). The response of the Ca^{2+} electrode was calibrated by addition of internal standards (Ca^{2+}-EGTA buffers) to the media. The consumption of oxygen was monitored with a Clark-type electrode (Yellow Springs Instruments). All figures describe the most representative experiments repeated at least three times and with two different RLM preparations.

Measurement of H_2O_2. Hydrogen peroxide was measured spectrophotometrically according to Cotton and Dunford [18]. This method is based on the oxidation of iodide by H_2O_2, catalyzed by horseradish peroxidase. Hydrogen peroxide formed by succinate-promoted mitochondrial respiration during 15 min (25 °C) was estimated in the supernatant after centrifugation of the incubation media (at 12000 × *g* for 3 min) to remove mitochondria.

Measurement of superoxide dismutase activity. The activity of the superoxide dismutase used in the experiments was measured according to Marklund [19]. The superoxide dismutase units appearing in the text are referred to as Marklund's units.

Results

Effect of δ-aminolevulinic acid in $\Delta\Psi$ and Ca^{2+} fluxes

Fig. 1A shows that isolated rat liver mitochondria preparations were able to maintain a succinate-promoted $\Delta\Psi$ of −195 mV as long as 20 min in the control incubation medium (pH 7.2). In the presence of 5 mM δ-aminolevulinic acid, mitochondria formed a lower $\Delta\Psi$ (−179 mV), which was disrupted after 10–12 min incubation. This effect was more pronounced with 10 and 15 mM δ-aminolevulinic acid: the $\Delta\Psi$(−155 and −104 mV) collapsed within 4 and 2–3 min, respectively. The mitochondrial $\Delta\Psi$ in 5 mM δ-aminolevulinic-acid-con-

taining medium in the presence of 2 μM catalase (an agent that inhibits δ-aminolevulinic acid autoxidation [10,12]) was essentially identical to that of the control. This suggested the involvement of δ-aminolevulinic acid-generated reactive intermediates, probably reactive oxygen species, in the process of $\Delta\Psi$ disruption.

Results similar to those observed for $\Delta\Psi$ were obtained by following Ca^{2+} fluxes (Fig. 1B). It is well known that Ca^{2+} uptake and retention by mitochondria are dependent on the membrane integrity [20]. In control experiments, mitochondria took up Ca^{2+} from the incubation media and retained it in the matrix throughout the observation period (20 min). In the presence of 5 mM δ-aminolevulinic acid, Ca^{2+} release occurred within 10–11 min. As in the case of $\Delta\Psi$, Ca^{2+} release occurs more rapidly in 10 mM δ-aminolevulinic-acid-containing medium (data not shown). Calcium fluxes in medium containing 5 mM δ-aminolevulinic

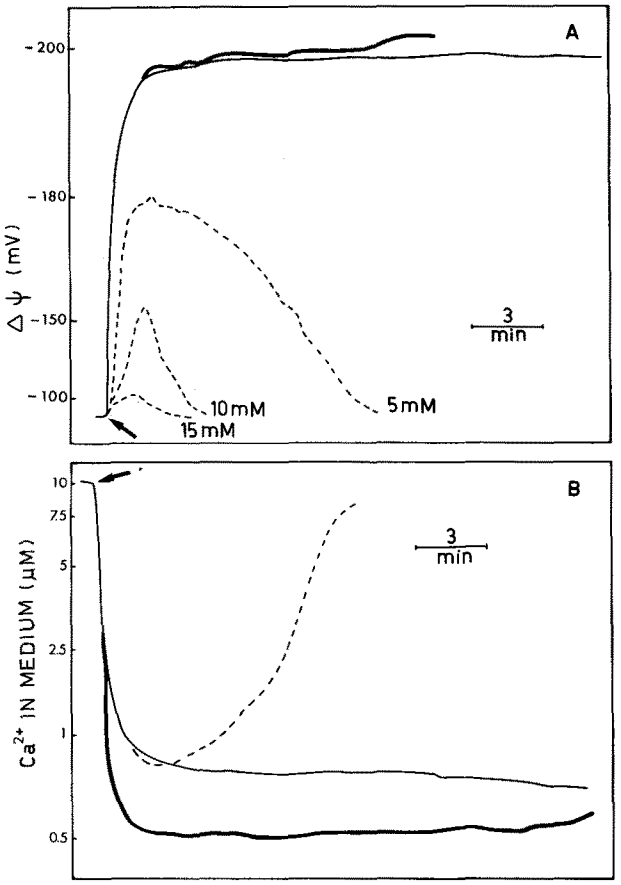

Fig. 1. Effect of 5–15 mM δ-aminolevulinic acid on $\Delta\Psi$ (A) and Ca^{2+} fluxes (B). Experiments were started with the addition of 1 mg/mL RLM (arrow) to 1 ml of the suspending medium containing 10 mM Hepes-Tris buffer (pH 7.2), 125 mM saccharose, 65 mM KCl, 2.5 mM succinate and 4 μM rotenone (plus 3 μM TPP^+ in A), at 25 °C (solid lines). Dashed lines show experiments performed in media containing 15, 10 (A) and 5 mM δ-aminolevulinic acid. Bold lines show runs with 5 mM δ-aminolevulinic acid in the presence of 2 μM catalase. Calcium fluxes and $\Delta\Psi$ in the presence of 2 μM catalase were the same as those shown by the bold lines.

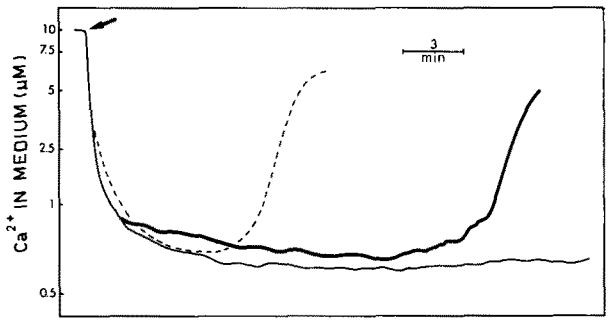

Fig. 2. Comparative effect of 20 μM H_2O_2 and 5 mM δ-aminolevulinic acid on Ca^{2+} fluxes. Experimental conditions of the control (solid line) were the same as in Fig. 1B. Dashed line and bold line show experiments carried out in media containing 5 mM δ-aminolevulinic acid and 20 μM H_2O_2, respectively.

acid plus 2 catalase were similar to those observed in control experiments. These results suggest that δ-aminolevulinic-acid-generated reactive intermediates might be responsible for Ca^{2+} release, probably by disrupting the mitochondrial membrane integrity and $\Delta\Psi$. In addition, the Ca^{2+} fluxes were measured in the presence of added H_2O_2 (Fig. 2). Hydrogen peroxide is formed in micromolar amounts by spontaneous δ-aminolevulinic-acid autoxidation [12]. After 15 min at 25 °C (pH 7.2), 5 mM δ-aminolevulinic-acid in normally aerated solutions produces 0.2 μM H_2O_2. It can be observed in Fig. 2 that, as compared to the 5 mM δ-aminolevulinic acid experiment, Ca^{2+} fluxes were only slightly affected by excess H_2O_2 (20 μM). These observations might indicate that H_2O_2 itself, generated by δ-aminolevulinic acid autoxidation, is not responsible for Ca^{2+} release. Participation of another δ-aminolevulinic-acid-generated reactive intermediates is analysed below.

Effect of o-phen and superoxide dismutase on δ-aminolevulinic acid-induced Ca^{2+} release

In order to investigate the involvement of the $OH^{\cdot}$ radical (or high valent oxo-metallic complexes [21]) in the process of mitochondrial damage, the effect of *o*-phen on the δ-aminolevulinic-acid-induced Ca^+ release was studied. Lesive $OH^{\cdot}$ radicals might be formed from δ-aminolevulinic acid-generated H_2O_2 and O_2^-, catalyzed by iron ions and/or other transition metals (the so-called Haber-Weiss reaction) present in the mitochondrial suspension. Three *o*-phen molecules form a chelate with one ferrous ion, modifying its redox potential and thus, preventing the occurrence of the Haber-Weiss reaction [22]. The chelation of iron by *o*-phen would also prevent the formation of high valent oxo-metalic complexes. Fig. 3 shows that 0.1 mM o-phen completely prevents the release of Ca^{2+} from the intramitochondrial matrix in 5 mM δ-aminolevulinic acid-containing medium (pH 7.2) during the observation period. In addition, in isotonic media (125 mM) con-

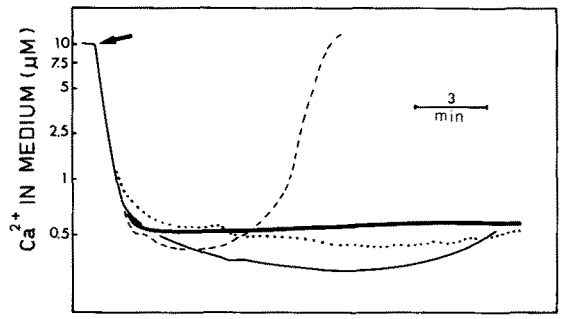

Fig. 3. Effect of *o*-phen and superoxide dismutase on δ-aminolevulinic acid-induced Ca^{2+} fluxes. Experimental conditions of the control (solid line) were as in Fig. 1B. Dashed line and bold line show experiments carried out in media containing 5 mM δ-aminolevulinic acid and 5 mM δ-aminolevulinic acid plus 0.1 mM *o*-phen. Calcium fluxes in the presence of 0.1 mM *o*-phen were the same as those represented by the bold line. The dotted line represents the effect of 50 units/ml superoxide dismutase on 5 mM δ-aminolevulinic acid-induced Ca^{2+} fluxes.

taining either sacarose or mannitol (both of which are $OH^{\cdot}$ scavengers) the effect of 5 mM aminolevulinic acid-induced damage was roughly identical to the control in KCl (data not shown). These results suggest that, unless $OH^{\cdot}$ radicals were formed in sites that are unaccessible to the scavengers, high-valent oxo-metallic complexes might be directly involved in the damage process. In addition, the presence of 50 units/ml superoxide dismutase in 5 mM δ-aminolevulinic-acid-containing medium has completely prevented the release of Ca^{2+} from intramitochondrial matrix (Fig. 3), whereas heat-inactivated superoxide dismutase had no effect whatsoever on the δ-aminolevulinic acid-promoted Ca^{2+} release. Since, as in the case of catalase, superoxide dismutase also inhibits δ-aminolevulinic acid autoxidation [10–12], δ-aminolevulinic acid-generated O_2^- might not be directly involved in the mitochondrial damage process.

Effect of δ-aminolevulinic acid in Ca^{2+} fluxes of pre-incubated mitochondria

Fig. 4 depicts the extent of Ca^{2+} flux changes at different periods of pre-incubation of mitochondria in media containing 2 mM δ-aminolevulinic acid (pH 7.2). Calcium uptake was initiated by addition of 5 mM succinate. The ability of mitochondria to retain Ca^{2+} decreased with increasing period of pre-incubation of mitochondria in 2 mM δ-aminolevulinic acid-containing media up to 30 min. Control experiments show that, within 30 min of pre-incubation, mitochondria were able to retain far more Ca^{2+} than in the presence of 2 mM δ-aminolevulinic acid. Pre-incubation of mitochondria for 30 min in medium containing 2 mM δ-aminolevulinic acid plus 2 μM catalase resulted in Ca^{2+} fluxes similar to those observed in the controls (data not shown). These data demonstrate that Ca^{2+} release from the intramitochondrial matrix is dependent on the period of previous exposure to δ-aminolevulinic-acid-generated reactive oxygen species.

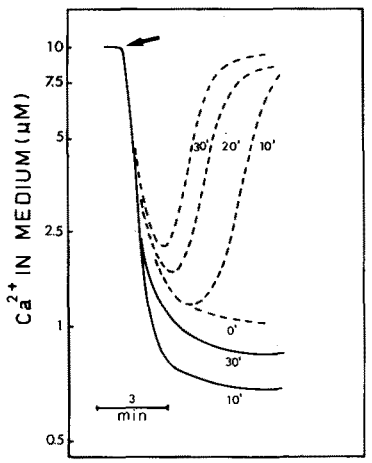

Fig. 4. Effect of 2 mM δ-aminolevulinic acid on Ca^{2+} fluxes of pre-incubated mitochondria. The suspending media containing 1 mg/ml RLM, 10 mM Hepes-Tris buffer (pH 7.2), 125 mM saccharose, 65 mM KCl, 4 μM rotenone and 2 mM δ-aminolevulinic acid (no δ-aminolevulinic acid in the control) were pre-incubated at 25° C for different time periods (10, 20, 30 min and no pre-incubation, as indicated). The Ca^{2+} fluxes were monitored after addition of 5 mM succinate (arrow) at 25° C. Dashed lines show experiments with 2 mM δ-aminolevulinic acid and solid lines the controls.

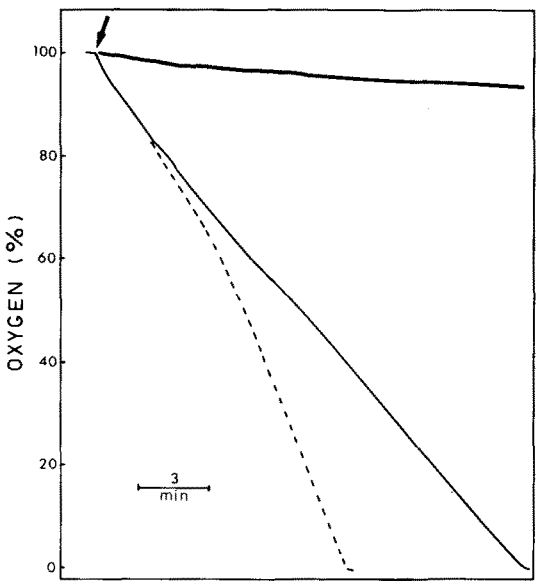

Fig. 5. Effect of 5 mM δ-aminolevulinic acid on the state-4 mitochondrial respiratory rate. The experiments (at 25°) were started (arrow) by addition of 2.5 mM succinate to 1 ml suspending medium containing 1 mg/mL RLM, 10 mM Hepes-Tris buffer (pH 7.2), 125 mM saccharose, 65 mM KCl, 4 μM rotenone and 5 mM δ-aminolevulinic acid (dashed line). The control contained no δ-aminolevulinic acid (solid line). The bold line depicts the oxygen uptake of 5 mM δ-aminolevulinic acid in the absense of mitochondria. The rate of oxygen uptake by 5 mM δ-aminolevulinic acid itself was not affected by the addition of mitochondria, in the absence of succinate.

Effect of δ-aminolevulinic acid on mitochondrial state-4 respiration

Fig. 5 shows that the presence of 5 mM δ-aminolevulinic acid caused a release of state-4 respiration supported by succinate oxidation (pH 7.2). The rate of oxygen uptake by 5 mM δ-aminolevulinic acid itself (by autoxidation [10–12]) in the absence of mitochondria was about 20-fold lower than that obtained from the difference between the rate of the δ-aminolevulinic-acid-induced mitochondrial respiration and the control rate. This demonstrated that the increase in oxygen uptake in δ-aminolevulinic-acid-containing media was not merely due to the oxygen consuming reaction of δ-aminolevulinic acid. These observations might indicate that δ-aminolevulinic acid-generated reactive oxygen species are able to increase the permeability of the mitochondrial inner membrane to H^+.

Discussion

Acute intermitent porphyria is an autosomal dominant disease, prevalent in Europe [2], in which the clinical manifestations are classically associated with δ-aminolevulinic acid accumulation and neurophysiological changes [2,3,6–8]. Interestingly, some of the symptoms of acute intermitent porphyria and lead poisoning are similar [1–3,23]. In the case of plumbism, direct participation of lead in the basic biochemical processes underlying the syndrome, such as lead-inhibition of several enzymes (e.g., brain adenyl cyclase) (Ref. 23 and references therein) and lead-stimulated iron-induced membrane lipoperoxidation [24], have also been proposed. On the other hand, the production of lesive reactive oxygen species from δ-aminolevulinic acid autoxidation, as the etiological agent in these disorders, was proposed by Bechara and co-workers [10–14]. In this context, we are now investigating the possible involvement of δ-aminolevulinic acid-generated reactive oxygen species in damage to cellular components.

The results described here strongly indicate that isolated rat liver mitochondria are damaged by δ-aminolevulinic-acid-generated reactive oxygen species. Thus, δ-aminolevulinic acid (2–15 mM) is able to disrupt the mitochondrial membrane potential (Fig. 1A), to promote Ca^{2+} efflux (Figs. 1B, 2, 3 and 4) and to release the state-4 mitochondrial respiratory rate (Fig. 5). The lesion was prevented by added catalase, superoxide dismutase or *o*-phen.

Catalase and superoxide dismutase have been shown to inhibit the δ-aminolevulinic acid autoxidation and to strongly reduce the formation of oxyradicals [10–12]. The slight induction of Ca^{2+} release by added 20 H_2O_2 (Fig. 2) demonstrates that the protective effect of catalase on the δ-aminolevulinic acid-induced damage is not due merely to H_2O_2 itself as the lesive species. Our inference that neither H_2O_2 nor O_2^- (generated by δ-aminolevulinic acid autoxidation [10–12]) are lesive to rat liver mitochondria is consistent with the proposal that such species are not sufficiently reactive to directly promote oxidation of cellular components [25]. Micromolar H_2O_2 produced by δ-aminolevulinic acid itself (0.2 μM in 15 min) and by mitochondrial respiration (3.4–3.6 μM in 15 min) accelerates δ-aminolevulinic acid autoxidation [12]. Added H_2O_2 (20 μM) failed to increase the 5 mM δ-aminolevulinic-acid-induced Ca^{2+} release (data not shown). Probably, the amount of H_2O_2 in the mitochondrial suspension is saturating with respect to δ-aminolevulinic acid autoxidation.

The protective effect of *o*-phen in the δ-aminolevulinic acid-induced Ca^{2+} release (Fig. 3) suggests the participation of transition metals in the damage process. These metals catalyze $OH^\cdot$ radical formation, but also can form highly oxidizing oxo- or peroxocomplexes [21]. The observation that neither saccharose nor mannitol prevents lesion to mitochondria (see Results) indicates either that $OH^\cdot$ radicals are not the lesive agents or that they are formed inside the mitochondria, impermeable to the scavengers. As described elsewhere for peroxidation of liposomes [26], $OH^\cdot$ radicals do not play the lesive role in this process.

In the case of $OH^\cdot$ radicals formed by the Haber-Weiss reaction between iron ions and δ-aminolevulinic-acid-generated H_2O_2 and O_2^- were directly involved in the mitochondrial damage, one should indeed expect abolition of the effects by both superoxide dismutase and catalase (see Figs. 2 and 3).

The δ-aminolevulinic-acid-generated reactive oxygen species are able to release the state-4 respiration (Fig. 5) and thus permeabilize the inner membrane of mitochondria to H^+. Several modes of lesion could be attributed to the uncoupling effect of δ-aminolevulinic-acid-generated reactive oxygen species. These include the oxidation and impairment of thiol proteins involved in the maintenance of membrane integrity and peroxidation of membrane phospholipids [26–29]. Damage to rat renal cortical mitochondria, promoted by reactive oxygen species generated by exogenous xanthine/xanthine oxidase, has also been observed [30]. In this case, an acceleration of state-4 respiration was also reported.

Hepatic mitochondrial damage was observed by electron microscopy of liver biopsy samples from acute intermittent porphyria patients [9]. High urinary δ-aminolevulinic acid levels were measured in these patients. The ultrastructural alterations reported in damaged mitochondria ranged from bizarre shapes to 'ghost' forms. The accumulation of fats [9] and the overload of iron [9,31] in the hepatocytes (of acute intermittent porphyria patients) were interpreted by Biempica et al. [9] as indirect evidence of impairment of mitochondrial functions, such as lipid β-oxidation and iron mobilization. The observed liver ferritin-Fe^{3+} over-

load [9] might be a potential source of 'free' Fe^{2+} when exposed to O_2^- [32] generated by δ-aminolevulinic acid autoxidation [10–12] and other sources [28]. Thus, one could connect the observed in vivo iron overload with the process described here of δ-aminolevulinic acid-induced mitochondrial damage, an iron-mediated process of reactive oxygen species generation.

Large lipofuscin bodies were observed in the hepatocytes of acute intermittent porphyria patients [9] and may result from oxidative stress-induced peroxidation of the membranes of mitochondria and other organelles [33]. In addition, α-ketoglutaraldehyde (the final product of δ-aminolevulinic acid autoxidation [11,12], which might form Schiff adducts with the membrane proteins [34]) together with lipoperoxidation-generated aldehydes might also lead to the lipofuscin-type products.

Although the range of δ-aminolevulinic acid concentration used in the present work (2–15 mM) is far above that found in vivo (ca. 0.01 mM in the blood plasma of one acute intermittent porphyria patient [35]), one must consider that: (i) following intraperitoneal injection of δ-aminolevulinic acid in rats, the drug accumulates in some organs (10-fold in liver, [5]); (ii) δ-aminolevulinic acid is a source of reactive oxygen species which initiates deleterious free radical reactions and; (iii) data on the intracellular compartimentalization of δ-aminolevulinic acid and its in situ effects are not available. Moreover, preliminary experiments have shown that 0.05 mM δ-aminolevulinic acid is able to promote ultrastructural changes in isolated rat liver mitochondria, by following mitochondrial swelling [27] observed via the light-cattering technique (Hermes-Lima, M. and Vercesi, A.E., unpublished data). Thus, it is tempting to propose, on the basis of the observed in vitro δ-aminolevulinic acid pro-oxidative effects that they may also be operative in vivo. In the case of lead poisoning, the reported damage to kidney mitochondria [1] might also be due to δ-aminolevulinic-acid-generated reactive oxygen species. Finally, we suggest the involvement of reactive oxygen species in the well-doccumented neuropathological manifestations common to both acute intermittent porphyria and lead poisoning.

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References

1 Chisolm Jr., J.J. (1971) Sci. Am. 224, 15–23.
2 Kappas, A., Sassa, S. and Anderson, K.E. (1983) The Metabolic Basis of Inherited Diseases (Stanbury, J.B., et al. eds.), pp 1301–1384, McGraw-Hill, New York.
3 Hindmarsh, J.T. (1986) Clin. Chem. 32, 1255–1263.
4 McGillion, F.B., Thompson, G.G., Moore, M.R. and Goldberg, A. (1974) Biochem. Pharmac. 23, 472–474.
5 McGillion, F.B., Thompson, G.G. and Goldberg, A. (1975) Biochem. Pharmac. 24, 299–301.
6 Brennan, M.J.W. and Cantrill, R.C. (1979) Nature 280, 514–515.
7 Brennan, M.J.W., Cantrill, R.C. and Kramer, S. (1980) Int. J. Biochem. 12, 833–835.
8 Gibson, J.B. and Goldberg, A. (1956) J. Pathol. Bacteriol. 71, 495–509.
9 Biempica, L. Kosower, N., Ma, M.H. and Goldfischer, S. (1974) Arch. Pathol. 98, 336–343.
10 Monteiro, H.P., Abdalla, D.S.P., Faljoni-Alário, A. and Bechara, E.J.H. (1986) Biochim. Biophys. Acta 881, 100–106.
11 Monteiro, H.P., Abdalla, D.S.P., Augusto, O. and Bechara, E.J.H. (1989) Arch. Biochem. Biophys. 271, 206–216.
12 Monteiro, H.P. (1986), PhD Thesis, Departamento de Bioquímica, Instituto de Química, Universidade de São Paulo, São Paulo, Brazil.
13 Medeiros, M.H.G., Marchiori, P.E. and Bechara, E.J.H. (1982) Clin. Chem. 28, 242.
14 Monteiro, H.P., Abdalla, D.S.P., Arcuri, A.S.A. and Bechara, E.J.H. (1985) Clin. Chem. 31, 1673–1676.
15 Fagian, M.M., Pereira da Silva, L. and Vercesi, A.E. (1986) Biochim. Biophys. Acta 852, 262–268.
16 Kamo, N., Muratsugu, N., Hongoh, R. and Kobatake, Y. (1979) J. Membr. Biol. 49, 105–121.
17 Muratsugu, M., Kamo, N., Kurihara, K. and Kabatake. J. (1977) Biochim. Biophys. Acta 464, 613–619.
18 Cotton, M.L. and Dunford, H.B. (1973) Can. J. Chem. 51, 582–587.
19 Marklund, S. (1976) J. Biol. Chem. 251, 7504–7507.
20 Åkerman, K.E.O. and Nicholls, D.G. (1983) Rev. Physiol. Biochem. Pharmacol. 95, 149–201.
21 Sutton, H.C. and Winterbourn, C.C. (1989) Free Rad. Biol. Med. 6, 53–60.
22 Nassi-Calo, L., Mello-Filho, A.C. and Meneghini, R. (1989) Carcinogenesis 10, 1055–1057.
23 Nathanson, J.A. and Bloom, F.E. (1975) Nature 255, 419–420.
24 Quinlan, G.J., Halliwell, B., Moorhouse, C.P. and Gutteridge, J.M.C. (1988) Biochim. Biophys. Acta 962, 196–200.
25 Sawyer, D.T. and Valentine, J.S. (1981) Acc. Chem. Res. 14, 393–400.
26 Minotti, G. and Aust, S.D. (1987) J. Biol. Chem. 262, 1098–1104.
27 Macedo, D.V., Ferraz, V.L., Pereira-da-Silva, L. and Vercesi, A.E. (1989) Integration of Mitochondrial Function (Lemasters, J. et al., eds.), pp. 535–542, Plenum, New York.
28 Sies, H. (1986) Angew. Chem. Int. Edn. Engl. 25, 1058–1071.
29 Kappus, H. (1985) Oxidative Stress (Sies, H., ed.), pp 273–310, Academic Press, New York.
30 Malis, C.D. and Bonventre, J.V. (1986) J. Biol. Chem. 261, 14201–14208.

31 Stein, J.A., Curl, F.D., Valsamis, M. and Tschudy, D.P. (1972) Am. J. Med. 53, 784–789.
32 Thomas, C.E., Morehouse, L.A. and Aust, S.D. (1984) J. Biol. Chem. 260, 3275–3280.
33 Sohal, R.S., Marzabadi, M.R., Galaris, D. and Brunk, U.T. (1989) Free Rad. Biol. Med. 6, 23–30.
34 Medeiros, M.H.G. and Bechara, E.J.H. (1986) Arch. Biochem. Biophys. 248, 435–439.
35 Gorschein, A. (1984) Biochem. J. 219, 883–889.