oxidative damage, much still needs to be explored about the mitochondrial production of reactive oxygen species and their contribution to signaling, aging and apoptosis. It is also hoped that MitoQ and related compounds can be used to explore whether these compounds have potential therapeutic effects. For example, the development of procedures to test these compounds in mouse models of human diseases is ongoing[8] but is beyond the scope of this chapter. The use of targeted coenzyme Q derivatives, in conjunction with increased understanding of their effects on mitochondrial function and redox behavior, may lead to new insights into the biology of mitochondria.

Acknowledgments

We thank Meredith F. Ross, Jordi Asin-Cayuela and Karim S. Echtay for helpful comments and discussions. This work was supported by grants from the Health Research Council of New Zealand, the Marsden Fund, administered by the Royal Society of New Zealand, and the Medical Research Council (UK).

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[4] The Mitochondrial Interplay of Ubiquinol and Nitric Oxide in Endotoxemia

By CONSTANZA L. LISDERO, MARIA CECILIA CARRERAS, ALAIN MEULEMANS, MARIANA MELANI, MICHEL AUBIER, JORGE BOCZKOWSKI, and JUAN JOSÉ PODEROSO

Introduction

Sepsis is a common cause of morbidity and mortality, particularly in the elderly, immune-compromised, and critically ill patients. Almost 25 years ago, we showed that clinical sepsis and septic shock were associated with acquired mitochondrial dysfunction; a critical inhibition of mitochondrial complex I was observed in skeletal muscle of patients with sepsis.[1] Recently, mitochondrial dysfunction was ascribed to mitochondrial

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0076-6879/04 $35.00

overproduction of reactive oxygen species and nitric oxide (NO), which may play a pivotal role in the pathophysiology of organ failure.[1–3]

Ubiquinone (UQ, coenzyme Q) is endogenously synthesized in every organ in a specific pathway branched from cholesterol biosynthesis[4]; organs have different ubiquinol content depending on the activity of this biosynthetic pathway.[5] In addition to its role as electron carrier in the mitochondrial respiratory chain,[6] ubiquinone exhibits prooxidant[7] and antioxidant[8] properties. Ubisemiquinone ($UQ^{\bullet -}$) participates in oxygen radical formation upon electron-transfer to oxygen,[9] whereas ubiquinol may be ascribed antioxidant properties upon its reaction with either peroxyl radicals or upon recovery of vitamin E radical.

In addition to the reversible inhibition of cytochrome oxidase, nitric oxide (NO) contributes to mitochondrial ubiquinol oxidation, within a reaction that yields $UQ^{\bullet -}$ and nitroxyl anion (NO^-) as well as oxygen- and nitrogen reactive species derived from $UQ^{\bullet -}$ autoxidation[10] (reactions [1–3]).

$$NO + UQH^- \rightarrow NO^- + UQ^{\bullet -} + H^+ \quad (1)$$

$$UQ^{\bullet -} + O_2 \rightarrow UQ + O_2^{\bullet -} \quad (2)$$

$$O_2^{\bullet -} + NO \rightarrow ONOO^- \quad (3)$$

The second-order rate constant for reactions [1–3] were calculated as 10^3–$10^4\ M^{-1}\ s^{-1}$,[10,11] $8 \times 10^3\ M^{-1}\ s^{-1}$, and $1.9 \times 10^{10}\ M^{-1}\ s^{-1}$, respectively. Reactions [1] and [3] are the most efficient routes for NO utilization in

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mitochondria and lead to the accumulation of peroxynitrite ($ONOO^-$), a species that modulates redox signaling and contributes to cell damage.[12] Accumulation of $ONOO^-$ has been associated with different pathological conditions, such as neurodegeneration[13] and sepsis.[3,14] Deleterious effects of $ONOO^-$ are likely a function of its strong oxidative capacity leading to protein oxidation and nitration.[12] In mitochondria, $ONOO^-$ may be scavenged by glutathione peroxidase or by direct interaction with electron donors, such as glutathione, NADH, or ubiquinol, present in either the matrix or the inner membrane[15] (reaction [4]). The reaction of $ONOO^-$ (as ONOOH) with UQH^- is first-order in $ONOO^-$ and zero-order in UQH^- indicating that it involves a *cage* rearrangement to give OH^- and NO_2^- (reaction [4])[15]:

$$ONOOH[OH^\bullet \ldots NO_2^\bullet] + UQH^- \rightarrow NO_2^\bullet + UQ^{\bullet -} + H^+ + OH^- \quad (4^*)$$

The generation of $UQ^{\bullet -}$ (reaction [4]) suggests that $ONOO^-$ may facilitate free radical propagation, supported by auto-oxidation of $UQ^{\bullet -}$ (reaction [2] following reaction [4]). Accordingly, we have shown that $ONOO^-$ increases O_2^- production in mitochondria.[15] However, increasing mitochondrial ubiquinol content clearly protects mitochondria from $ONOO^-$ effects.[15] This suggests the occurrence and significance of radical-radical termination reactions among the terms of reaction [4] and involving the NO_2^-/NO_2^-, $UQ^{\bullet -}/UQ$, and OH^-/OH^- redox pairs. These termination reactions intercept effects of aggressive radicals on mitochondrial components. For instance, tyrosine nitration by $ONOO^-$ proceeds in two steps: (a) H-abstraction by $NO_2^\bullet$ or $OH^\bullet$ to form tyrosyl radical, and (b) tyrosyl radical reaction with $NO_2^\bullet$, to form nitrotyrosine. Therefore, although mitochondrial production of reactive oxygen species and $ONOO^-$ are related to ubiquinol concentration (*pro-oxidant* effects),[8,9] the features of reaction [4] support an *antioxidant* activity of ubiquinol. Reactions [1–4] in mitochondria have important implications for the regulation of the levels of NO in the mitochondrial matrix and production of oxyradicals, processes intimately associated with mitochondrial integrity and function.

At physiological matrix NO levels (20 to 40 n*M*) with about 2 to 3 nmol ubiquinone/mg mitochondrial protein,[11] this sub-system is likewise capable of removing all NO in excess. In this setting, almost all NO is consumed to form O_2^-, and its Mn-SOD-mediated dismutation product

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*Alternatively, $ONOOH\ [OH^\bullet \ldots NO_2^\bullet] + UQH^- \rightarrow NO_2^- + UQ^{\bullet -} + H^+ + OH^\bullet$

H_2O_2.[11,16] However, at high and sustained matrix NO concentration (≈500 n*M*), these reactions could not afford the cost of NO detoxification. In the context of mitochondrial derangement in septic shock, an important point to consider is the source of mitochondrial NO. It is noteworthy that iNOS is induced by inflammatory mediators in different organs and in macrophages. We have recently reported an increase in mitochondrial protein nitration during iNOS induction in *E. coli* endotoxemia and described the functional correlation between the loss of diaphragmatic force and mitochondrial respiratory impairment.[3,17] Moreover, recent data showed that NO can be synthesized within mitochondria by a mtNOS[18–20] that could be activated during sepsis; accordingly, Boveris *et al.* and Escámes *et al.* reported an increase of mtNOS activity in rat endotoxemia.[21,22]

This chapter provides the methodological bases that support *(a)* the relation between UQ content, mitochondrial NO steady-state concentration and peroxynitrite-mediated mitochondrial damage in representative organs and *(b)* the contribution of mtNOS to peroxynitrite formation in mitochondria.

Experimental Model: Endotoxemic Animals and Sample Preparation

Endotoxemic Animals

Sprague-Dawley male albino rats (weight: ~350 g) from Charles River France Inc. were divided into 2 groups, which received either sterile 0.9%, NaCl (control animals) or 10 mg/kg *E. coli* endotoxin suspension in NaCl (serotype 0.26 B6; DIFCO, Detroit, MI), intraperitoneally (septic animals). Animals were sacrificed 6 hours after LPS inoculation and liver, lung, diaphragm and heart were excised. Liver of normal C57BL/6J mice and transgenic mice deficient in iNOS gene C57BL/6-*Nos*2^{tm1Lau} from Jackson Laboratories (Bar Harbor, ME) were also utilized. Animals were maintained on a 12-hr light/dark cycle with free access to food and water and were fasted one night before the experiment with water *ad libitum*.

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Homogenate and Mitochondrial Preparations

Frozen tissue samples were homogenized with an Ultraturrax T25 (Janke and Kunkel, IKA Works, Cincinnati, OH) in lysis buffer (50 m*M* Tris HCl [pH 7.4], 0.1 m*M* EDTA, 1 μM leupeptin, 1 μM PMSF, 1 μM aprotinin). The crude homogenates were centrifuged at 4000*g* for 20 min at 4°, and stored at −70° until use. Mitochondria were isolated as previously described[20]; excised organs were placed in an ice-cold homogenization medium (1 g/10 ml) consisting of 100 m*M* KCl, 50 m*M* Tris-HCl, 1 m*M* EDTA, 5 m*M* $MgCl_2$, 1 m*M* ATP (pH 7.2) at 4° for isolation of diaphragm mitochondria and 0.23 *M* mannitol, 70 m*M* sucrose, 10 m*M* Tris-HCl, 1 m*M* EDTA (medium A) for isolation of liver, heart, and lung mitochondria. Mitochondria were purified by Percoll gradient centrifugation and finally resuspended in 0.25 *M* sucrose at a 20 mg/ml protein concentration.

Sources of NO in Endotoxemia: iNOS Expression and Activity

Measurement of NOS Expression and Activity

Proteins of tissue homogenates (100 μg/lane) and mitochondria (50 μg/lane) were separated by electrophoresis on precast 7.5% SDS-polyacrylamide gels (Bio-Rad, Richmond, CA) and transferred to a PVDF membrane (Bio-Rad). The membranes were incubated with a rabbit anti-mouse iNOS polyclonal antibody (1:1000) (Transduction Laboratories, Lexington, KY), and blotted with a goat anti-rabbit IgG (1:3000) conjugated to alkaline phosphatase (Bio-Rad), followed by detection of immunoreactive proteins by a chemiluminescence method (Bio-Rad). *E. coli* LPS-stimulated rat alveolar macrophages obtained by bronchoalveolar lavage were used as positive controls.

NOS activity was determined by the conversion of ^{3}H-L-arginine to ^{3}H-L-citrulline as follows: mitochondrial and homogenate activities were measured in 50 m*M* potassium phosphate buffer, pH 7.5 in the presence of either 100 μM (mitochondria) or 20 μM (homogenates) L-arginine, 0.1 μM ^{3}H-L-arginine, 0.1 m*M* NADPH, 0.3 m*M* $CaCl_2$, 0.1 μM calmodulin, 10 μM BH_4, 1 μM FAD, 1 μM FMN, 50 m*M* L-valine and 0.1 mg protein. Specific activity is calculated by subtracting the remaining activity in the presence of 50-fold excess concentration of the NOS inhibitor L-NMMA. Calcium-independent NOS activity is measured in the presence of 2 m*M* EGTA.

Tissue homogenates from LPS-treated animals expressed iNOS protein, detected in the Western blot analysis as an homogeneous band

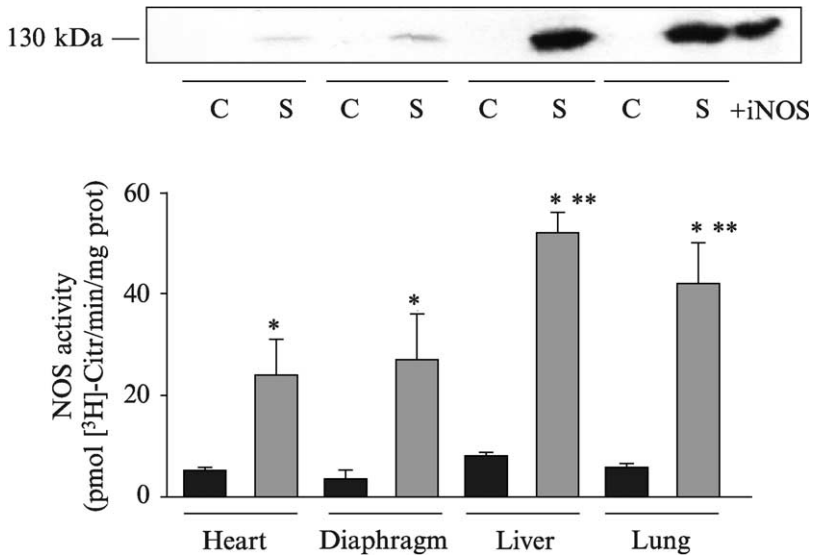

FIG. 1. iNOS expression and Ca^{2+}-independent activity of rat tissue homogenates in endotoxemia. Proteins were separated by electrophoresis in 7.5% SDS-PAGE and iNOS protein was detected with a rabbit anti-mouse iNOS polyclonal antibody (1:1000); *denotes $p < 0.05$; *C*, control; *S*, septic. *$p < 0.001$ *vs* controls by Student *t* test; **$p < 0.05$ *vs* endotoxemic heart by ANOVA and Dunnett test.

with molecular identity respect to control iNOS expressed by *in vitro* LPS-stimulated rat alveolar macrophages (Fig. 1). The level of expressed iNOS was ten-fold higher in liver and lung than in heart and diaphragm homogenates. iNOS specific activity resulted about two-fold higher in liver and lung than in diaphragm or cardiac muscle (Fig. 1). These data agree with early reports of Szabó *et al.*[23] in endotoxic shock.[24]

iNOS is Translocated to Mitochondria

Considering the recent reports on the presence of Ca^{2+}-dependent nNOS-α in liver mitochondria (mtNOS[25]), we analyzed a putative contribution of this enzyme to matrix NO concentration in liver tissue during endotoxemia. Immunoblotting analysis of mitochondrial proteins against

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anti-nNOS antibodies confirmed an mtNOS band at 130 kDa, as previously described.[25] In addition, septic samples exhibited a lower expression of this constitutive mtNOS isoform and a higher expression of iNOS respect to the control samples (Fig. 2A, $p < 0.02$). Reciprocally, calcium-dependent mtNOS activity was 50% lower in mitochondria from septic animals than in the control ones, while calcium-independent activity, which was absent in the control samples, increased markedly during endotoxemia (more than 100% of total NOS activity) (Fig. 2B). The selective modulation of each specific NOS isoform in septic samples avoids the possibility of a contamination with cytosolic proteins and also excludes the contribution of the cross-reactivity of the utilized antibodies. Accordingly, immunoprecipitation of mitochondrial proteins with anti-iNOS antibodies showed that iNOS protein translocates to mitochondria in endotoxemia (Fig. 2C). Moreover, after endotoxemia, iNOS expression increased in cytosol and mitochondria of wild type but not in mice with iNOS gene disruption (Fig. 2D).

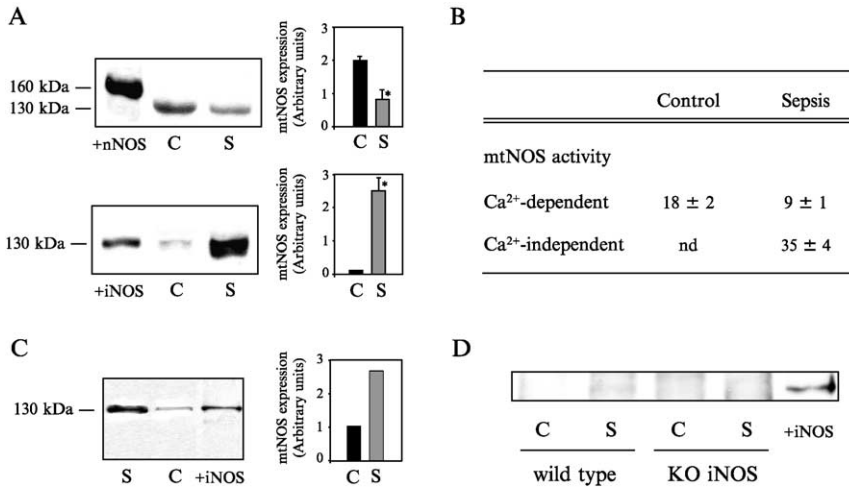

FIG. 2. Mitochondrial expression and activity of liver NOS isoforms in endotoxemia. (A) Proteins were separated as in Fig. 1 and revealed with monoclonal anti-nNOS antibodies and polyclonal anti-iNOS antibodies. The respective densitometries are placed on (A), right, and NOS activities in (B). In (C), it is shown as a representative Western blot of immunopurified mitochondrial proteins. In (D), mitochondria from controls and iNOS gene deficient mice. Abbreviations as in Fig. 1. $^{*}p < 0.02$ *vs* controls and $^{**}p < 0.01$ *vs* Ca^{2+}-dependent by Student *t* test.

These results indicate that, *(a)* matrix NO concentration is differentially contributed by both constitutively expressed mtNOS and increased cytosolic iNOS translocated to mitochondria and *(b)* targeted iNOS protein could be the main source of matrix NO in endotoxemia.

The Role of Ubiquinol in Endotoxemia

Ubiquinone Content and NO-Induced Hydrogen Peroxide Production by Mitochondria

This was performed as previously described[25]: 5 to 10 mg of mitochondrial protein was homogenized at 4° with 4 volumes (v/w) of water in an Ultraturrax for 20 sec. One ml of the homogenate was poured into a test tube containing 7 ml of a mixture of ethanol-*n*-hexane (2:5), and the tube was rapidly shaken for 10 minutes to extract UQ. This extraction was repeated three times, and the combined n-hexane layer was evaporated to dryness under a stream of nitrogen. The resulting residue was dissolved in 0.2 ml of ethanol and subjected to HPLC. Total ubiquinone determination was performed with reverse-phase chromatography as carried out in a Hypersil C-18 column (15 cm × 4.6 mm I.D., 5 μm) (Hypersil, England). The mobile phase was prepared by dissolving 6.1 g of $NaClO_4$. H_2O in 1000 ml of ethanol-methanol-70% $HClO_4$ (700:300:1). The flow rate was 1.2 ml/min. The HPLC measurements were performed at 30°. The HPLC system consisted of a 510 pump (Waters, France) with an automatic injector (717 Autosamples, Waters). The UV detector for measuring oxidized ubiquinone was a photodiode array detector (996, Waters). For chromatography, 10 μl of the extracts or standards were injected into the apparatus; retention times were 4.3 min for UQ-*9* and 5.3 min for UQ-*10*, determined at 275 nm with UV detector.

HPLC analysis of ubiquinone extracted from mitochondria (UQ-*9* + UQ-*10*) showed that heart organelles have the highest content (3.7 ± 0.5 nmol/mg mitochondrial protein), liver and diaphragm contain intermediate amounts of ubiquinone (1.7 ± 0.3 and 1 ± 0.2, respectively) and lung samples had the lowest content (0.14 ± 0.03) (Fig. 3A). The mitochondrial ubiquinone content of tissues from septic animals was similar to that of organelles from the control ones (not shown).

The mitochondrial H_2O_2 production rate was continuously monitored by the horse-radish (HRP)/p-hydroxyphenyl acetic acid (p-HPA) assay[16] with a Hitachi F-2000 fluorescence spectrophotometer (Hitachi Ltd.; Tokyo, Japan) supplemented with 12 U/ml HRP, 250 μM p-HPA, at 0.1 to 0.5 mg protein/ml, and with excitation and emission wavelengths of

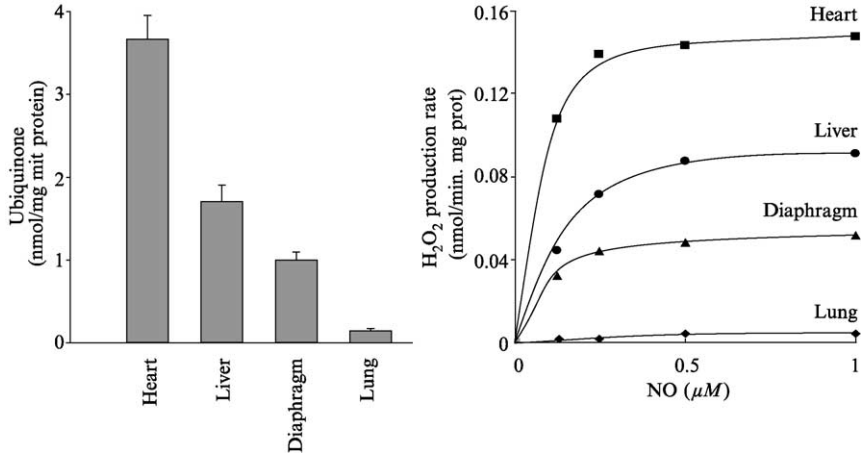

FIG. 3. NO-dependent mitochondrial H_2O_2 production rate and ubiquinol content. (A) Mitochondrial total ubiquinone of rat tissues, as measured by HPLC. (B) Hydrogen peroxide production rate of mitochondria in the presence of crescent NO concentration. Data are different from each other by two-factor ANOVA.

315 and 425 nm, respectively. The utilized medium consisted of 0.23 *M* mannitol, 70 m*M* sucrose, 30 m*M* Tris-HCl, 5 m*M* Na_2HPO_4/KH_2PO_4, and 1 m*M* EDTA (pH 7.4) with 8 m*M* succinate as substrate; to assess maximal mitochondrial H_2O_2 production at complex III, antimycin was added at 2 nmol/mg protein in selective experiments.

The mitochondrial H_2O_2 production rate was achieved at 0.1 to 1 μM NO; nitric oxide solutions (1.2 to 1.8 m*M*) were obtained by bubbling NO gas of 99.9% purity (AGA GAS Inc., Maumee, OH) in water degassed with He for 30 min at room temperature and stored for a week at 4°. Maximal H_2O_2 yield was achieved at 0.25 μM NO; H_2O_2 production rate was highest in heart organelles followed by liver, diaphragm and lung (Fig. 3B). According to reactions [1] and [2] and after Mn-SOD catalyzed dismutation, differences in NO-dependent H_2O_2 production rates of mitochondria from the different tissues were strictly related to their respective ubiquinone content (Fig. 4). A similar relationship was obtained when heart submitochondrial particles depleted of ubiquinone were subsequently reconstituted with known concentrations to achieve a similar ubiquinone content as the membranes from the analyzed rat tissues.[26] Submitochondrial particles were resuspended in 0.15 *M* KCl at a concentration of 20 mg protein/ml and lyophilized for 9 h to completely

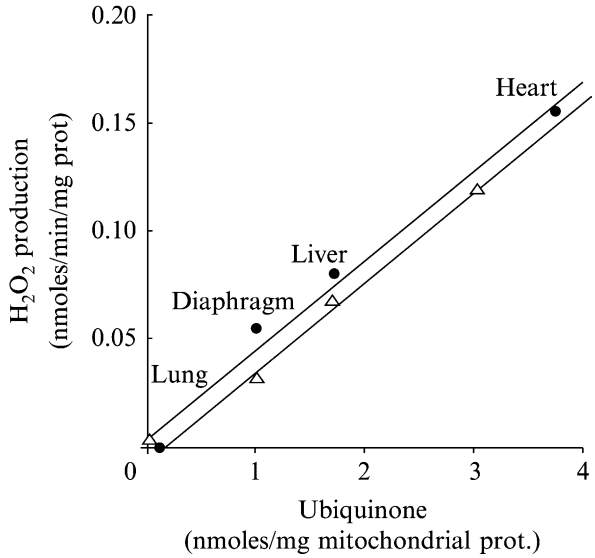

FIG. 4. Correlation between NO-dependent hydrogen peroxide production and ubiquinone content. (●), H_2O_2 production of isolated mitochondria from the different tissues in the presence of 1 μM NO (△): H_2O_2 production of heart submitochondrial particles depleted of endogenous ubiquinone and reconstituted with UQ-*10* at same NO concentration (both, $r^2 = 0.98$, $p = 0.011$ and 0.009, respectively).

dehydrate the samples. Mitochondrial ubiquinone was removed by suspending the lyophilized particles in n-pentane by gentle homogenization, and the suspension was shaken in a glass-stoppered tube for 5 min at 0°, 5 times. Extracted ubiquinone was 4 to 5 nmol UQ/mg protein. To incorporate ubiquinone at different concentrations, the depleted particles were gently homogenized in a small volume of n-pentane (1 to 2 ml) containing UQ-*10* at a concentration of 50 to 100 nmol/mg protein, and the suspension was shaken in an iced bath for 30 min. The particles were centrifuged, dried by evaporation for one hour and stocked. Depleted particles showed a markedly decreased oxygen uptake and NADH cytochrome *c* reductase activity. These parameters were normalized after reconstitution of mitochondrial UQ-*10*. The UQ-*10* content of ubiquinone-reconstituted particles (1 to 5 nmol/mg mitochondrial protein)

[26] F. Aberg, E-L. Appelkvist, G. Dallner, and E. Lars, *Arch. Biochem. Biophys.* **295,** 230 (1992).

was determined by HLPC with electro-chemical detection with an amperometric detector.

In the presence of 1 μM NO, mitochondrial H_2O_2 production rates of organelles with spontaneous or exogenously manipulated ubiquinone content were closely similar and thus, both, resulted well correlated with ubiquinol content ($r = 0.98$, $p < 0.05$) (Fig. 4).

The Endotoxemic Mitochondrial Damage

Mitochondrial Activities

Rats treated with LPS showed a significant decrease of the respiratory control ratio (state 3 O_2 uptake/state 4 O_2 uptake rates) of mitochondria from liver (−28%) and diaphragm (−35%) ($p < 0.05$), while in heart and lung mitochondria, this index resulted slightly modified (Table I). A lowering of respiratory control ratio is consistent with a partial uncoupling of oxidative phosphorylation to electron transfer rates that is currently associated to mitochondrial impairment. Moreover, these results reveal tissue-specific endotoxemic effects on the different mitochondrial populations.

Mitochondrial Hydrogen Peroxide Production

Mitochondria from endotoxemic animals supplemented with antimycin showed an increased mitochondrial H_2O_2 production rate reflecting a higher intramitochondrial concentration of superoxide radical, the product of the univalent reduction of oxygen.[9] After endotoxemia, the mitochondrial production rate of H_2O_2 was significantly increased in diaphragm (+ 100%) and liver mitochondria (+34%), with respect to their

TABLE I
MITOCHONDRIAL RESPIRATORY CONTROL RATIO IN ENDOTOXEMIA

	Heart	Diaphragm	Liver	Lung
Control	4.7 ± 0.3	4.5 ± 0.4	3.6 ± 0.3	2.8 ± 0.9
LPS	3.8 ± 0.3	2.9 ± 0.2*	2.6 ± 0.2*	2.9 ± 0.5

Data are expressed as mean ± S.E.M of samples from 5–7 animals by duplicate;
*denotes significantly different from respective control group by Student's t test ($p < 0.05$).

TABLE II
MITOCHONDRIAL H_2O_2 PRODUCTION RATE IN ENDOTOXEMIA

	Heart	Diaphragm	Liver	Lung
Control	0.31 ± 0.02	0.13 ± 0.02	0.15 ± 0.01	n.d.
LPS	0.32 ± 0.01	0.24 ± 0.03*	0.18 ± 0.01*	n.d.

Maximal H_2O_2 production rate was determined fluorometrically in the presence of 6 m*M* succinate as substrate and 2 μM antimycin as complex III inhibitor. Data are expressed in nmol/min/mg protein as the mean ± S.E.M of samples from 7 animals by duplicate;

* denotes significantly different from respective control group by Student *t* test ($p < 0.05$).
n.d.: not detected.

respective controls ($p < 0.05$, Table II). No differences were assessed in heart mitochondria; H_2O_2 could not be detected in lung organelles. The increased rate of H_2O_2 production of antimycin-supplemented mitochondria is consistent with selective functional impairment of organelles in the compromised tissues and indicates the previous inhibition of electron transfer rate at mitochondrial complexes II–III.

Detection of $ONOO^-$ in Mitochondria

Presence of $ONOO^-$ was accomplished by detection of 3-nitrotyrosine residues in mitochondrial proteins by Western blot with a monoclonal anti-nitrotyrosine antibody (Upstate Biotechnology Incorporated, Lake Placid, MA) developed by Beckman and co-workers.[27] In brief, 60 μg of proteins of the mitochondrial suspension were separated by electrophoresis on precast 7.5% SDS-polyacrylamide gel (Bio-Rad, Richmond, CA), transferred to a nitrocellulose membrane (Bio-Rad, Richmond, CA) and revealed by using the anti-nitrotyrosine antibody. Bovine serum albumin (BSA) nitrated after 30 minutes of incubation with 1 m*M* SIN-1, was used as a positive control. Incubation of the antibody with 10 m*M* nitrotyrosine, prior to the membrane incubation, was used to ensure the specificity of the antibody.

Western blot analysis of mitochondrial homogenates from endotoxemic animals exhibited a reproducible pattern of protein nitration for several

[27] J. S. Beckman, Y. Z. Ye, P. Anderson, J. Chen, M. A. Accavetti, M. M. Tarpey, and C. R. White, *Biol. Chem. Hoppe-Seyler* **375,** 81 (1994).

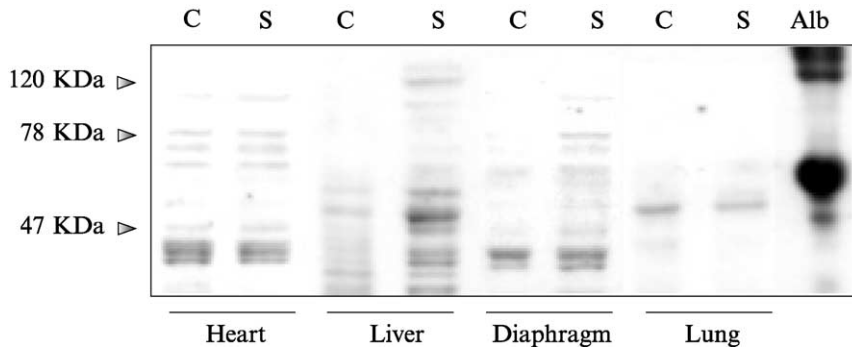

FIG. 5. Mitochondrial protein nitration. Nitration of mitochondrial proteins after 6 h of endotoxemia. *C*, control, *S*, septic. *Alb*, bovine serum albumin nitrated after 30 min exposure to 1 m*M* SIN-1.

bands (Fig. 5). Interestingly, immunoblot intensity was highest in liver and diaphragm mitochondria and lowest in heart samples, which showed only weak bands similar to control samples. No protein nitration was observed in lung mitochondria. The preincubation of the anti-nitrotyrosine antibody with free nitrotyrosine significantly attenuated the blotting signal, thus ensuring the specificity of the antibody (not shown).

Considering that increases in tissue and mitochondrial [NO]ss depend on iNOS activity and mitochondrial translocation, it is expected that in rat endotoxemia liver and lung would have twice the [NO]ss of heart and diaphragm. In diaphragm in similar conditions, [NO]ss was reported to be 0.473 μM.[3] Assuming a similar [NO]ss in heart and 0.9 μM in liver and that $d[ONOO^-]/dt/d[H_2O_2]/dt$ ratio increases at higher [NO]ss, being about 6 at 0.5 μM NO,[11] and considering that measured mitochondrial $d[O_2^-]/dt$ and $d[H_2O_2]/dt$ depends on [NO]ss and ubiquinol concentration (reactions [1] and [2]; Figs. 3 and 4), $d[ONOO^-]/dt$ should be higher in heart than in liver or diaphragm. Thus, very low nitration in heart organelles at high $d[ONOO^-]/dt$, may indicate the significance of high ubiquinol in heart protection as surmized from reaction [4]. In contrast, low ubiquinol in lung mitochondria decreases $d[O_2^-]/dt$ and $ONOO^-$ formation (reaction [1–3]), thus limiting mitochondrial protein nitration (Fig. 5).

The results show that, *(a)* in endotoxemia, oxidative stress, and the production of reactive oxygen species depend on increased NO production as released by iNOS; *(b)* relative amounts of NO production and ubiquinol

concentrations determine distinct H_2O_2 and peroxynitrite production rates; and *(c)* intermediate concentrations set UQ-centered reactions in rates that favor subsequent impairment of mitochondrial respiratory functions by nitration of mitochondrial proteins. It is worth noticing that the highest cytosolic iNOS levels are followed by a proportional increase of mitochondrial iNOS, which should enhance the vectorial release of NO to the matrix.

These concepts are in agreement with the improvement in liver biochemical parameters of endotoxemic rats previously treated with UQ-*10*.[28] Administration of UQ-*10* decreased the overall mortality of endotoxemic dogs.[29] On the basis of iNOS activity/UQ content ratios, it is estimated that 3 to 10 fold increased UQH^- would be required in diaphragm and liver to limit critical $ONOO^-$ effects. It is noteworthy that the rates of the sequential reactions [1] and [2] depend on the levels of reduced UQ rather than total UQ in membranes. It is accepted that normal rat heart has a low degree of reduction of ubiquinone (30 to 40%).[24] However, UQH^-/UQ ratio is markedly increased in heart mitochondria in endotoxemia because of NO inhibition of cytochrome oxidase which augments the level of reduced intermediaries on the substrate-side of the electron transfer chain.

In this model of endotoxemia, the occurrence and pattern of nitration in different tissues is consistent with the clinical features and the evolution of septic multiorganic failure. Liver, skeletal muscle and diaphragm are compromised early in the evolution of sepsis, while heart compromise occurs at a later stage[23] or is less related to NO increase.[30] Endotoxemic lung distress is probably not related to primary mitochondrial damage. This fact suggests that lung damage, a common finding in clinical and experimental sepsis, is a consequence of neutrophil and macrophage recruitment and activation[31] rather than a result of early mitochondrial impairment. Although in those situations associated with excessive NO production a therapeutic utilization of UQ-*10* could be suggested, the magnitude and efficiency of the potential protection will depend on tissue bioavailability, incorporation to the mitochondrial membranes, and redox condition of ubiquinol.

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[5] Mitochondrial Respiratory Chain Dysfunction Caused by Coenzyme Q Deficiency

By PIERRE RUSTIN, ARNOLD MUNNICH, and AGNÈS RÖTIG

Introduction

Coenzyme Q_{10} (CoQ_{10}; ubiquinone 50) is an extremely hydrophobic molecule that plays a critical role in both antioxidant defenses and electron transfer activity of cell membranes, especially in the respiratory chain located in the mitochondrial inner membrane.[1,2] CoQ_{10} content varies strongly between tissues. Human heart, kidney, liver (114, 66.5, 55 μg/g tissue respectively) and intestine, colon, testis or lung (11.5, 10.7, 10.5, 7.9 μg/g tissue respectively) CoQ_{10} contents differ by more than one order of magnitude.[1] Skeletal muscle, pancreas, thyroid, spleen and brain (40, 33, 24.7, 24.6, 13.4 μg/g tissue respectively) show median values. In addition, decreased ubiquinone content with age has been reported, although the decrease is variable depending on the tissue studied.[1] On the other hand, the redox status of CoQ_{10}, rather than its absolute amount, may be the crucial parameter to be examined under most physiological conditions.[3] On one hand, decreased CoQ_{10} content and/or increased oxidation status might denote a slowdown of oxidative metabolism activity in tissues, therefore a decreased need for electron transfer and antioxidant capacities, especially with age. On the other hand, decreased CoQ_{10} content might be one of the causative events in a cascade leading to the decrease of oxidative and antioxidant capacity with age. In other words, CoQ_{10} decrease over time could be seen either as a positive adjustment to a decreased metabolic demand, or as a harmful progressive deficiency with age.

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