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Review

Nitric oxide, complex I, and the modulation of mitochondrial reactive species in biology and disease

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Abstract

Mitochondria are the specialized organelles for energy metabolism but also participate in the production of O_2 active species, cell cycle regulation, apoptosis and thermogenesis. Classically, regulation of mitochondrial energy functions was based on the ADP/ATP ratio, which dynamically stimulates the transition between resting and maximal O_2 uptake. However, in the last years, NO was identified as a physiologic regulator of electron transfer and ATP synthesis by inhibiting cytochrome oxidase. Additionally, NO stimulates the mitochondrial production of O_2 active species, primarily O_2^- and H_2O_2, and, depending on NO matrix concentration, of $ONOO^-$, which is responsible for the nitrosylation and nitration of mitochondrial components. By this means, alteration in mitochondrial complexes restricts energy output, further increases O_2 active species and changes cell signaling for proliferation and apoptosis through redox effects on specific pathways. These mechanisms are prototypically operating in prevalent generalized diseases like sepsis with multiorgan failure or limited neurodegenerative disorders like Parkinson's disease. Complex I appears to be highly susceptible to $ONOO^-$ effects and nitration, which defines an acquired group of mitochondrial disorders, in addition to the genetically induced syndromes. Increase of mitochondrial NO may follow over-expression of nNOS, induction and translocation of iNOS, and activation and/or increased content of the newly described mtNOS. Likewise, mtNOS is important in the modulation of O_2 uptake and cell signaling, and in mitochondrial pathology, including the effects of aging, dystrophin deficiency, hypoxia, inflammation and cancer.

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Abbreviations: eNOS, iNOS, mtNOS and nNOS, endotelial, inducible, mitochondrial and neuronal nitric oxide synthases; H_2O_2, hydrogen peroxide; Mn-SOD, manganese-superoxide dismutase; NO, nitric oxide; NOS, nitric oxide synthase; O_2^-, superoxide radical; $ONOO^-$, peroxynitrite; $UQH^{\cdot}$, ubisemiquinone

Keywords: Nitric oxide; Complex I; mtNOS physiology; mtNOS pathology; Mitochondriopathies; Parkinson's disease; Sepsis; Cancer

Contents

1. Mitochondria and the mitochondrial production of superoxide radicals

Mitochondria are thermodynamically organized to provide electron transfer from substrates to O_2 ($E^{o\prime}$ = NADH, −0.32 V and O_2, +0.82 V). Oxyreductive reactions involving the inner membrane components are associated to proton pumping and a membrane potential ($\varphi \sim 0.15$ V) is generated and further dissipated through ATP synthase activity. The regulation of electron flow and coupled oxidative phosphorylation depends on mitochondrial O_2 concentration and ADP/ATP ratio; increased ADP is representative of low cell energy levels. In this context, mitochondria continuously move in a bi-directional transition between a quiet resting respiratory condition (state 4 O_2 uptake, 10–40 ngat O/min mg prot), and an active respiratory

state in the presence of ADP (state 3 O_2 uptake, 80–250 ngat O/min mg prot). Due to very low cytochrome *c* oxidase K_M for oxygen (0.5–2 μM), by many years, mitochondrial respiration was thought to follow an "all or nothing" paradigm with the implicit statement that, up to very low O_2 levels, respiration does not depend on O_2 availability and that electron transfer was rather modulated by redox pairs content.

On the other hand, in pioneer works it was discovered that mitochondria reduce O_2 univalently, leading to the formation of O_2^- and H_2O_2 (Boveris and Chance, 1973; Boveris and Cadenas, 1975). This process is part of normal respiration; 2% of utilized O_2 is normally driven to the formation of O_2^- and its Mn-SOD mediated dismutation product, H_2O_2.

Isolation of mitochondrial components led to identify five multimeric complexes (complexes I–V) embedded in the inner membrane. Non-enzymatic reactions of O_2^- formation occurs at complex I and at complex III and likely both depend of autooxidation of increased intermediary ubisemiquinone (UQH·), at specific ubiquinone pools, as emerged from the use of rotenone (complex I inhibitor at PSSP protein) and antimycin (complex III inhibitor at cytochrome b_H).

2. The mitochondrial complex I

Mitochondrial complex I (NADH: ubiquinone oxidoreductase) catalyzes electron transfer from NADH to ubiquinone, is composed by about 40 units (Walker, 1992) and contains FMN and iron–sulphur centers. Seven polypeptides of complex I are encoded by mitochondrial genes (ND 1, 2, 3, 4, 4L, 5, and 6). Topological distribution of complex I components are schematized in Fig. 1; in a sequential order, main complex I components include 54 kDa flavoprotein, 24, 75 and 49 kDa proteins, and proteins TYKY and PSST bound to inner membrane which transfer electrons to ubiquinone; two complex I-linked ubiquinone pools have been detected (Raha and Robinson, 2000). The electron transfer rate in complex I is relatively high; NADH oxidation proceeds at 250–500 nmol/min mg prot, as compared with the

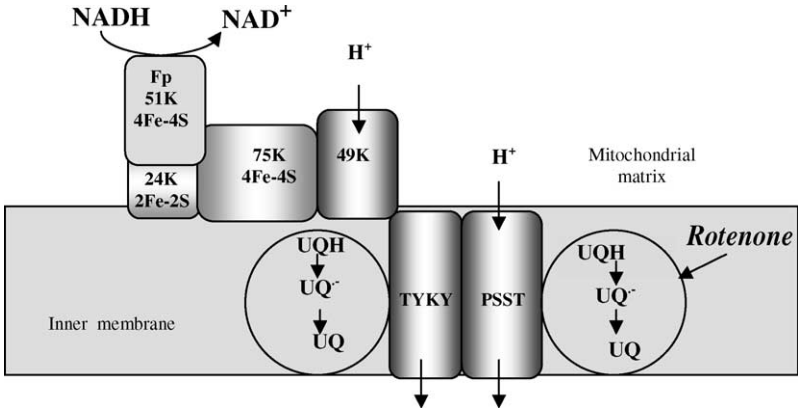

Fig. 1. Scheme of the structure of complex I (modified from Raha and Robinson, 2000).

electron transfer rate of ubiquinol: cytochrome *c* reductase, 100–150 nmol/min mg prot.

3. Inhibition of complex I, cell cycle, signaling and apoptosis

Changes in respiratory-chain activity modify cell cycle activity and cell duplication. Agents that increase mitochondrial biogenesis as etoposide or genistein allow cells to come into differentiation. In contrast, the number of immature mitochondria in stem cells is low allowing maximal cell proliferation and self-renewal. Non-selective and controlled decrease of respiratory rate is associated to proliferation in normal and tumoral cells (Galli et al., 2003). In contrast, selective and partial electron transfer inhibition elicits opposite effects. In this context, complex I inhibitor rotenone lowers basal hepatocyte proliferation and decreases the incidence of hepatocellular carcinoma (Wang et al., 1999). Considering the low K_M for O_2 of cytochrome oxidase, it is not surprising that rat muscle mitochondria respiration is limited after 80% rotenone-inhibition of complex I (Rossignol et al., 2003). Accordingly, respiration of 143B isolated cells began to decrease at 40% rotenone-inhibition of complex I with a simultaneous increase of the production of reactive oxygen species. Moreover, rotenone induced a significant decrease of cell growth at ~60% complex I inhibition, with a threshold value of ~35% cell respiratory rate decay (Barrientos and Moraes, 1999). The evidences propose that cell arrest and apoptosis (a hyper-differentiation state) are related to the production of O_2 active species, rather than to mitochondrial respiration (Seaton et al., 1997).

Different extra-cellular stimuli have been shown to increase the production of mitochondrial reactive O_2 species, affecting redox balance and signaling cascades (Borutaite and Brown, 2003); effects of tumor necrosis factor (TNFα), activation of c-Jun N terminal kinase (JNK) and down-regulation of mitochondrial extra-cellular ERK1/2 (Alonso et al., in press) are likely mediated by mitochondrial ROS formation.

4. The mitochondrial effects of nitric oxide

The discovery of NO by Ignarro and Moncada opened a broad perspective in vascular regulation and pharmacology and also allowed to explore its intracellular effects. Since 1994, several groups, including our, analyzed mitochondrial NO utilization and function.

Nitric oxide reversibly binds to Cu^{2+}–B center of cytochrome oxidase and inhibits electron transfer to O_2 and respiration in rat skeletal muscle, liver and heart mitochondria (Cleeter et al., 1994; Brown, 1995; Poderoso et al., 1996, 1999b; Cassina and Radi, 1996; Wolin et al., 1999; Brookes et al., 2003). Similar effects were described in perfused rat heart (Poderoso et al., 1998). NO-dependent inhibition of O_2 uptake is achieved at low and physiological NO concentrations; 50–100 nM NO inhibits by half cytochrome oxidase activity. Additionally, NO acts on other mito-

chondrial components as well; at 0.3–0.5 μM NO inhibits electron transfer between cytochromes *b* and c_1 (Poderoso et al., 1996); in addition, as we reported for the first time, exposure of rat liver or skeletal muscle mitochondria to NO markedly increases the production rate of O_2^- and H_2O_2 (Poderoso et al., 1996). At physiological 20–50 nM NO concentrations, most mitochondrial NO is utilized in the formation of O_2^- and H_2O_2 (Poderoso et al., 1999b). These effects rely on the inhibition of cytochrome oxidase and complex III and on direct reactions of NO with membrane ubiquinol, which increases the steady-state concentration of UQH·. At higher NO concentrations, NO and derived O_2^- react very fast and NO-utilization is mainly driven to the formation of $ONOO^-$ (Poderoso et al., 1999a), the powerful oxidant and nitrating agent (Radi et al., 2002). Under physiological conditions, controlled formation of NO sets up cytochrome oxidase activity and matrix steady-state $ONOO^-$ concentration. In this condition, the relatively low $ONOO^-$ yields are detoxyfied by direct or radical-mediated reactions with mitochondrial components like ubiquinol (Schöpfer et al., 2000) or NADH (Valdez et al., 2000); the existence of a stable low $ONOO^-$ concentration is indicated by detection of small amount of nitrotyrosine in the normal organelles (Lisdero et al., in press). At high NO concentration however, $ONOO^-$ production overwhelms the utilizing reactions and oxidation/nitration of lipids and proteins may impair mitochondrial functions. Protein oxidation and nitration may have a capital importance in cell cycle. Oxidized and nitrated proteins are preferentially targeted for fast proteolytic degradation. Several proteolytic enzymes responsible for degrading oxidized proteins decline with age, implying a less efficient removal and an accumulation of oxidized proteins in aging. In addition, recent evidence suggest that mitochondrial proteins are nitrated and denitrated in respiring mitochondria (Aulak et al., 2004). Thus, although protein nitration is usually understood as a cumulative and damaging process in which nitrotyrosine-containing proteins lose activity and impair cell function, it may be also selective, dynamic, and reversible, being both vital and detrimental (Fig. 2).

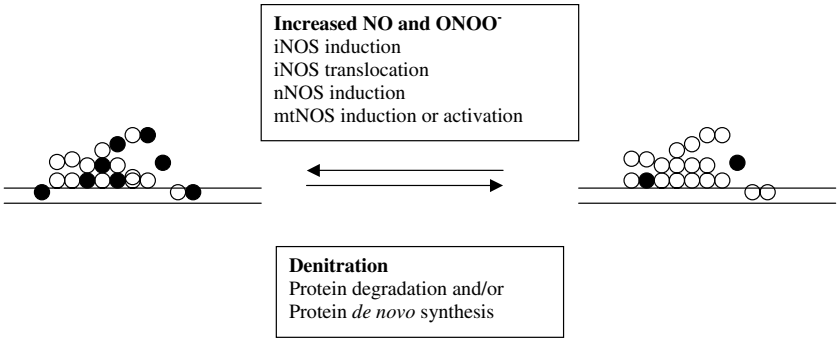

Fig. 2. Scheme of membrane-bound and matrix mitochondrial proteins with no or low nitration (open circles) and the reversible transition to high nitration (dark circles) dynamically driven by molecular collisions and enzyme activities.

5. Mitochondrial NOS in normal physiology and pathology

In the last decade, different investigators gave evidence of the existence of NOS in mitochondria (mtNOS) (Ghafourifar and Richter, 1997; Giulivi et al., 1998). Recent studies indicated that mtNOS is a variant of nNOS α (Riobó et al., 2002) constitutively localized in the inner mitochondrial membrane. As mtNOS catalyzes NO production within mitochondria, the variations of mtNOS expression and activity have marked effects on mitochondrial functions, such as O_2 uptake and energy gain, the production of O_2^- and H_2O_2, and cell signaling. Our group reported that mtNOS activity depends on thyroid status and that a marked increase of mtNOS content and activity provides a functional explanation for the down-regulation of O_2 uptake in hypothyroidism (Carreras et al., 2001). In accord, we found increased nitrated mitochondral proteins in hypothyroid mitochondrial (Fig. 3A). An increased mtNOS content was also detected in rat adaptation to prolonged cold environment with consequent effects on the insulating protection against hypothermia (Peralta et al., 2003). In accord to the NO effects on electron transfer, mtNOS activity correlates with the production of H_2O_2 rat liver during development (Fig. 3B), indicating that the NO-dependent cytosolic steady-state concentration of diffusible H_2O_2 operates on proliferation, cell cycle arrest and apoptosis. In brain and liver development, mtNOS activity is very low in embryos with high cell proliferation rate and low H_2O_2 and increases in the postnatal life associated to tissue quiescence with low proliferation rate and high H_2O_2 (Fig. 3B; Riobó et al., 2002; Carreras et al., 2003). In

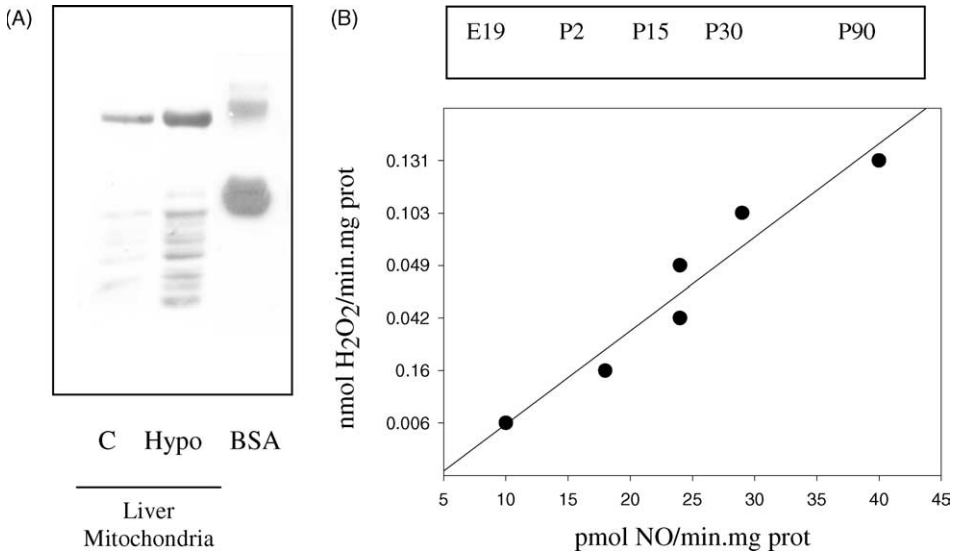

Fig. 3. (A) Nitration of liver mitochondrial proteins in rat hypothyroidism (Hypo) with high mtNOS content as compared with normal controls (C) and nitrated bovine albumin (BSA). (B) Linear correlation between mtNOS activities and mitochondrial H_2O_2 production rates in rat liver development from embryonic stage E19 to postnatal P2-P90.

addition, activation of mtNOS can be followed by release of cytochrome *c* and apoptosis (Ghauforifar et al., 1999). Moreover, NO stimulates mitochondrial biogenesis, which associates to cell differentiation (Nisoli et al., 2004).

The constitutive NO production by mtNOS changes the NO/O_2 ratio and affects cytochrome oxidase activity, these relationships link mitochondrial O_2 uptake and O_2 availability, which otherwise are not interdependent, up to critical hypoxic levels. Likewise, this effect should explain the NO-dependent increased mitochondrial efficiency in terms of O_2 utilization and ATP production in hypoxia.

Finally, it has been reported that mitochondrial NO acts as an antioxidant by inhibiting the oxidation of mitochondrial lipids and proteins and protecting membranes from high oxidative non-modulated rates (Paxinou et al., 2001).

6. Nitric oxide and mitochondrial complex I

Specific effects of NO on complex I have been reported in the last years. Clementi et al. (1998) observed that NO at μM concentrations for long periods inhibits respiration and complex I activity. In accord, we reported that long-exposure to NO induced the production of O_2^- and $ONOO^-$ in heart and liver mitochondria resulting in persistent inhibition of NADH: cytochrome *c* reductase activity, while complexes II and III remained unaffected (Riobó et al., 2001b). Tyrosine nitration of complex I proteins was prevented by SOD and uric acid and specifically attributed to mitochondrial O_2^- and $ONOO^-$ generation. Cooperation between NO and calcium during ischemia was described by Jekabsone et al. (2003); NO and calcium caused loss of mitochondrial cytochrome *c* and started caspase 3-dependent apoptosis. On the basis of the reversibility of NO-inhibition of complex I by reduced thiols or light it was proposed that the process involves S-nitrosation or Fe-nitrosylation (Jekabsone et al., 2003). A less reversible inhibitory effect is more consistent with an $ONOO^-$-induced complex I nitration that mimics rotenone action (Riobó et al., 2001b). In accord, Murray et al. (2003), in an elegant study, reported the $ONOO^-$-dependent nitration sites of complex I from bovine heart mitochondria, deduced from MS/MS fragmentograms and ion chromatograms. The most reactive site in subunit B14 was Tyr122, while the most reactive region in B15 contained three closely spaced tyrosines: Tyr46, Tyr50, and Tyr51.

Considering that NO increases the mitochondrial O_2^- production rates of complexes I and III, it remains unclear why the first is more selectively sensitive to $ONOO^-$; it is possible that the large pool of ubiquinol at complex III protects protein from nitration (Schöpfer et al., 2000).

7. Complex I and disease: the hereditary mitochondriopathies

Mitochondria contain their own DNA (mtDNA) and the machinery for synthesizing RNA and proteins; mtDNA has only 37 genes and thus, most of mitochondrial proteins are encoded by nuclear DNA and imported from cytosol. Different

respiratory-chain disorders associated to relatively low-specificity clinical syndromes have been described (Di Mauro and Schon, 2003). The defects are caused by mutations in mtDNA or nDNA. Mutations in mtDNA NADH-dehydrogenase (ND) genes lead to LHON, MELAS and Leigh's syndromes. Mutation in complexes II–III genes may also produce Leigh's syndrome and different encephalomyopathies, cardiomyopathy and exercise intolerance; complex II defects may induce tumors, like paraganglioma or pheochromocytoma. Other neurological and hematological syndromes arise on defects in cytochrome oxidase (COX) I–III mitochondrial genes. Mutations in nDNA have been only reported in subunits of complexes I and II but disorders may result from mutations in ancillary proteins required for assembly in the inner membrane or for insertion of cofactors.

Since defective mitochondria generate more reactive oxygen species, primarily O_2^- by auto-oxidation and electron leaks, it was hypothesized that oxidative stress is an additional factor for mitochondrial disease (Wei et al., 2001). This hypothesis has been supported by the findings that oxidative stress and oxidative damage in tissues and culture cells are increased in elderly subjects and patients with mitochondrial diseases. Cybrids with MELAS (mitochondrial encephalomyopathy) point mutation in the tRNALeu (UUR) gene of mtDNA with high proportions of mutant mtDNA and low respiratory rate, markedly increased peroxide production when exposed to 95% O_2. The appearance of apoptotic cells was high in cybrids with impaired respiratory function due to the mutation. The antioxidant acetyl cysteine suppressed both peroxide production and apoptosis, indicating a causal relationship between peroxide production and apoptosis in cells carrying the mtDNA mutation (Zhang et al., 1998). In addition, transgenic strains of *Caenorhabditis elegans* for homologue of NDUFV1 gene encoding a subunit of NADH-ubiquinone oxidoreductase exhibit phenotypic features of complex I deficiency like lactic acidosis and decreased NADH-dependent respiration and are hypersensitive to oxidative stress in accord to decreased antioxidant defenses (Grad and Lemire, 2004). Moreover, activation of the apoptotic cascade and increased release of cytochrome *c* into the cytosol was detected in LHON cybrid cells bearing the 3460/ND1 and 14484/ND6 mutations; thus, cybrids with complex I deficiency are sensitized to an apoptotic death through a mechanism involving mitochondria (Ghelli et al., 2003).

8. Complex I and disease: the acquired mitochondriopathies. Parkinson's disease

Parkinson's disease is an important cause of morbidity and disability in the adult population, characterized by a selective apoptotic loss of dopaminergic neurons in the tier ventral of the *pars compacta* of the substantia nigra. Although the cause of nigral apoptotic death is not completely defined, the occurrence of mitochondrial damage and oxidative stress has been repeatedly found in the clinical setting and in experimental models (Fahn and Cohen, 1992). Decreased complex I activity has been found in neural and extra neural tissues and circulating cells. In accord, cybrid cell lines created using mtDNA of Parkinson's disease patients showed complex I deficiency and increased production of reactive oxygen species (Swerdlow et al., 1996).

Recently, the role of NO in Parkinson's disease has gained significance by the finding of 3-nitrotyrosine in the core of Lewy bodies, the pathological hallmark of this neurodegenerative disorder (Good et al., 1998), and of nNOS over-expression in the brain of patients with Parkinson's disease (Eve et al., 1998). In the same way, we observed nNOS over-expression and 3-nitrotyrosine in circulating neutrophils from patients with this disease, suggesting a generalized deregulation of the nNOS gene (Gatto et al., 2000). The presence of nitrotyrosine, a "footprint" of peroxynitrite formation was also observed in mitochondria from rats with experimental Parkinson's disease induced by the toxic MPTP and its activated ion MPP+ (1-methyl-4-phenylpyridinium) which could be prevented by previous administration of 7-nitroindazole, a relatively specific nNOS inhibitor. In addition, nNOS gene deficient mice are more resistant than wild-type animals to toxic effects of MPTP. Moreover, in SH-SY5Y neuroblastoma cells, MPTP and its active product MPP+, increase the mitochondrial production of NO, suggesting an activating effect on mtNOS associated to Bax increase, release of cytochrome *c* and caspase activation (Dennis and Bennett, 2003). While 144 kDa mtNOS (neuronal variant) is high at early developmental stages in parallel to the course of brain and cerebellum structural plasticity, adult brain mtNOS expression is considerably lower than that of neonates (Riobó et al., 2002). Hence, pathological increases of mtNOS activity in adulthood may contribute to neurodegeneration. The data indicates that an increased matrix NO level, secondary to nNOS over-expression or mtNOS activation, leads to increased production of O_2^- and complex I alteration, and turns normal cell signaling to the apoptotic condition. In accord, oxidative stress induced by complex I inhibition with 1-methyl-4-phenylpyridinium activated biphasically the pro-apoptotic stress-activated c-Jun N-terminal kinase (JNK) and the early transcription factor NF-κB in SH-SY5Y neuroblastoma cells (Cassarino et al., 2000).

It is not clear why substantia nigra is particularly susceptible to the NO toxic effects that occur in Parkinson's disease leading to the neurodegeneration of dopaminergic-catecholaminergic cells. Substantia nigra has high concentrations of 6-hydroxydopamine that readily reacts with NO ($k = 1.5 \times 10^4$ $M^{-1} s^{-1}$) leading to the formation of dopamine semiquinone and increasing O_2^- and $ONOO^-$ formation (Riobó et al., 2001a).

9. Sepsis and endotoxemia

Sepsis is a common cause of morbidity and mortality, particularly in the elderly, immuno-compromised and critically ill patients. Almost 25 years ago, we showed that clinical sepsis and septic shock were associated with acquired mitochondrial dysfunction; a marked inhibition of complex I activity was observed in skeletal muscle mitochondria of septic patients (Poderoso et al., 1978). In addition, we reported decreased respiratory rates and increased mitochondrial production of O_2^- and H_2O_2 in skeletal muscle from rats with experimental sepsis due to cecal ligation and perforation (Peralta et al., 1993), and in diaphragm and liver from endotoxemic animals (Boczkowski et al., 1999; Lisdero et al., in press).

Recently, mitochondrial dysfunction was confirmed in the experimental and clinical field (Brealey et al., 2002) and septic damage was attributed to an energy failure secondary to NO-induced ATP depletion. This notion agrees with the spontaneous presence of nitrotyrosine in liver and diaphragm mitochondria of endotoxemic rats with high NO steady-state levels (Boczkowski et al., 1999; Lisdero et al., in press) and with the deleterious effects of mitochondrial $ONOO^-$ in tissue functions, as impairment of muscle contraction (Boczkowski et al., 1999, 2001).

The main source of NO in endotoxemia is iNOS, as induced by the cooperative effects of inflammatory cytokines (Boczkowski et al., 1999; Lanone et al., 2000). However, increased mtNOS activity was detected by Boveris et al. (2002) in endotoxemic rats. Moreover, we detected translocated iNOS in mitochondria with high Ca^{2+}-independent activity, differently to that of the constitutive neuronal-type mtNOS (Lisdero et al., in press). The findings indicate that cytosolic or mitochondrial NO induces a high production of O_2^-, which at high NO concentrations (0.3–0.5 μM) is 80% driven to formation of $ONOO^-$ (Poderoso et al., 1999b), which in turn nitrates mitochondrial components and, particularly, the highly susceptible complex I. Low rates of electron transfer through complex I critically restricts ATP synthesis and contributes to self-sustaining increased O_2^- production and further damage. Given the ubiquity of NO induction in sepsis, most organs and systems are subjected to restricted energy supply, and to pathologic cell signaling that ends in high protein degradation, poor cell renewal and apoptosis, which are the cellular basis of septic multiorgan failure. To some extent, septic mitochondrial failure may be compared with mitochondrial genetic disorders. In both groups, several organs and tissues are affected indicating a common basic

Table 1
Symptoms and clinical findings common to sepsis and mitochondrial genetic disorders

Central nervous system	*Kidney*
Dementia	Tubular acidosis
Stroke	Renal failure
Convulsions	Glomerulopathies
Psychosis	Pancytopenia
Ataxia	Sideroblastic anemia
Heart	*Endocrine and metabolic*
Cardiac dilatation	Diabetes mellitus
A–V conduction disorders	Hypothyroidism
Tachycardia	Adrenal insufficiency
Lung	Metabolic acidosis
Respiratory insufficiency	Lactic acidosis
Dyspnea	*Gastrointestinal*
Respirator winning failure	Liver failure
Diaphragm paralysis	Hepatitis
Exercise intolerance	Nausea
Skeletal muscle	Vomiting
Muscle weakness	Constipation
Fatigue	Intestinal pseudo-occlusion
	Pancreatitis

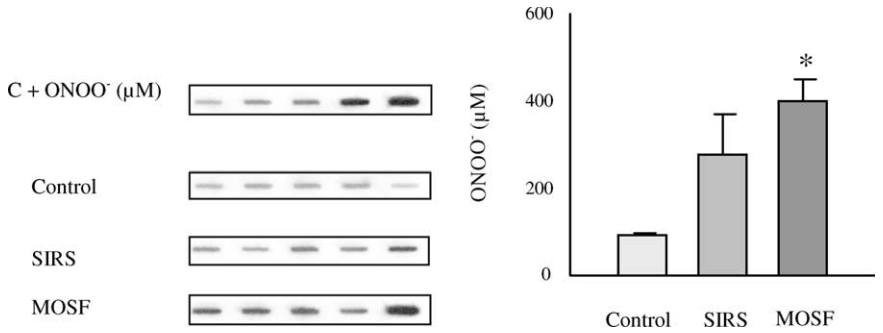

Fig. 4. Detection of 3-nitrotyrosine residues in plasma proteins from septic patients with systemic inflammatory response syndrome (SIRS, initial stage) and with multiple organ septic failure (MOSF, late stage), in the left panel. Calculated $ONOO^-$ concentration for exposed plasma proteins in the different conditions, in the right panel.

pathogenic mechanism (Table 1). Nitration of circulating plasma proteins in septic patients reflects the magnitude of the process and correlates well with the clinical condition and organ impairment (Fig. 4).

10. Cancer

In the last years, the role of NO in tumor biology has gained significance; iNOS has been consistently found in solid tumors, and it was proposed that NO promotes tumor growth. However, NO also shows antitumor activity by inhibiting proliferation, promoting differentiation and reducing the metastatic spread of some tumor cell types. As reported by our group and others, cumulative evidence showed that H_2O_2, a product of NO-regulation of mitochondrial function and cell redox state are clearly related to normal growth and to activation of signaling kinases (Carreras et al., 2003) as well as to cell transformation and cancer (Galli et al., 2003).

Tumor cells exhibit generalized defects in mitochondrial functions; low rates of electron transfer and poorly coupled ATP synthesis correlate with faster tumor growth and high invasive behavior (Simonnet et al., 2002). This effect contrasts with the critical inhibition of complex I occurring at high matrix NO that induce cell cycle arrest and apoptosis. In a recent work, we reported that most activities of tumor and proliferating normal mitochondria are uniformly maintained at 20–30% of those of quiescent organelles and that, in connection with down-regulated electron transfer, proliferating and tumor mitochondria only retain 20–50% of the maximal H_2O_2 production rate of adult organelles and no more than 5% of mtNOS activity (Galli et al., 2003). On these bases it is surmised that, differently to segmental or partial electron transfer inhibition, a generalized reduction of mitochondrial functions and activities resulting in a fixed and non-modulated cell H_2O_2 could constitute a general platform to sustain tumor proliferation and to impede cell progression to growth arrest and apoptosis.

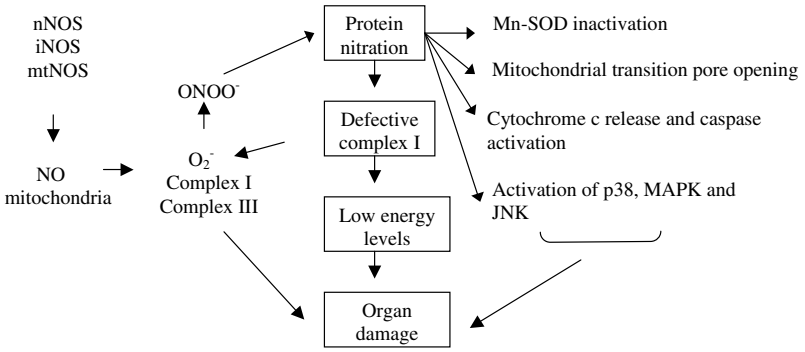

Fig. 5. Scheme representing mechanisms of organ damage consecutive to mitochondrial NO increased steady-state concentrations.

11. Concluding remarks

Mitochondrial NO, originated in mitochondria or the cytosol, has important effects on mitochondrial functions. Inhibition of cytochrome oxidase activity by mtNOS-produced NO is likely critical to energy availability during the cell cycle and to adapt the respiration rate to hypoxic conditions. Likewise, mtNOS is implicated in the control of respiration by thyroid hormones and in the adaptation to a cold environment. On the other hand, canonical NOS variants produce NO that reach mitochondria after sorting cytosolic components as myoglobin, NADH and GSH, depending on the duration of eNOS and nNOS activation and of the NO pulses, on the expression of iNOS and nNOS, and on the putative translocation of NOS isoforms by anchoring proteins, i.e. dystrophin or caveolin III. A main consequence of NO regulation is the production of O_2^- by complexes I and III. Complex I is very susceptible to genetic or acquired NO-mediated damage; increased O_2^- production by complex I perpetuates organ damage in prevalent pathologies by energy failure, oxidative-nitrosative stress, and by turning normal cell signaling to pathways that activate apoptosis (Fig. 5).

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