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Analysis of the Pathways of Nitric Oxide Utilization in Mitochondria

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The regulatory role that mitochondria play in cell dysfunction and cell-death pathways involves the concept of a complex and multisite regulation of cellular respiration and energy production signaled by cellular and intercellular messengers. Hence, the role of nitric oxide, as a physiological regulator acting directly on the mitochondrial respiratory chain acquires further relevance. This article provides a survey of the major regulatory roles of nitric oxide on mitochondrial functions as an expression of two major metabolic pathways for nitric oxide consumption: a reductive pathway, involving mitochondrial ubiquinol and yielding nitroxyl anion and an oxidative pathway involving superoxide anion and yielding peroxynitrite. The modulation of the decay pathways for nitrogen-and oxygen-centered radicals is further analyzed as a function of the redox transitions of mitochondrial ubiquinol. The interplay among these redox processes and its implications for mitochondrial function is discussed in terms of the mitochondrial steady-state levels (and gradients) of nitric oxide and superoxide anion.

Keywords: mitochondria, nitric oxide, peroxynitrite, cytochrome oxidase, oxygen radicals, nitrogen radicals, peroxynitrite

INTRODUCTION

The regulatory role that mitochondria play in cell dysfunction and cell-death pathways[1–5] has been extensively investigated in recent years and, not surprisingly, a subset of human diseases involving mitochondrial dysfunction has been well recognized. Neurodegenerative diseases, such as Alzheimer's, Parkinson's, and Huntington's disease involve mitochondrial defects at different respiratory chain complexes and deletions or point mutations on mtDNA are found in different mitochondrial encephalomyopathies[5]. A few features of the mitochondrion are of relevance in connection with the role mentioned above: first, the mitochondrion is an energy-transducing organelle; second, it is the major cellular source of H_2O_2, which originates from $O_2^{\cdot -}$ formed by the electron-transfer chain and, third, the adenine nucleotide translocase (ANT) mitochondrial activity -related to energy coupling and the mitochondrial membrane permeability pore[6]- functions as a sensor of oxida-

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tive stress and is apparently associated with mitochondrial functional changes involved in the signaling of apoptosis cascades. Hence, the delicate relationship between mitochondria and apoptosis requires consideration of ATP levels[2] as well as the steady-state of O_2 radicals.

The concept of a complex and multisite regulation of cellular respiration and energy production signaled by cellular and intercellular messengers has evolved in the last few years and is still under development. The classical and elegant concept of regulation of mitochondrial O_2 uptake by ADP considers that energy needs drive respiration and that availability of ADP exerts the kinetic control of respiration and energy production over a wide range of O_2 concentrations. A new concept on regulation of cellular respiration has been recently advanced, which retains the notion from the classical concept that energy demands drive respiration but it places the kinetic control of both respiration and energy supply on the availability of ADP to F_1-ATPase and of O_2 and ·NO to cytochrome oxidase[7]. In this way, ·NO, in addition to its role as intercellular messenger in diverse physiological processes, acquires an essential role as a mitochondrial regulatory metabolite. In this connection, the reversible inhibition of cytochrome oxidase by ·NO[8,9] and its competitive character with molecular O_2 suggest that ·NO may become the first known physiological regulator to act directly on the mitochondrial respiratory chain.

This article provides a survey of the major regulatory roles of ·NO on mitochondrial functions as an expression of two major metabolic pathways for ·NO consumption: a reductive pathway, involving mitochondrial ubiquinol and yielding nitroxyl anion (NO^-) and an oxidative pathway involving $O_2^{\cdot -}$ anion and yielding peroxynitrite ($ONOO^-$). The modulation of the decay pathways for nitrogen- and oxygen-centered radicals is further analyzed as a function of the redox transitions of mitochondrial ubiquinol. The interplay among these redox processes and its implications for mitochondrial function is discussed in terms of the mitochondrial steady-state levels (and gradients) of ·NO ($[\cdot NO]_{SS}$) and O_2·-($[O_2^{\cdot -}]_{SS}$).

MITOCHONDRIA REDUCTIVE DECAY PATHWAYS OF NITRIC OXIDE

The reductive pathways accounting for ·NO decay follow the general electron transfer in equation 1 with production of nitroxyl anion. Perhaps the most significant biological process encompassing

$$\cdot NO + e^- \rightarrow NO^- \quad [1]$$

the ·NO → NO^- transition in cytosol is the interaction of ·NO with Cu, Zn-superoxide dismutase (reaction 2)[11], whereas in mitochondria is the interaction of ·NO with with respiratory chain ubiquinol (reaction 3)[12]. Reaction 3 proceeds at modest rates (k_3= ~2 × 10^3 $M^{-1}s^{-1}$) with a stoichiometry of ·NO consumed *per* ubiquinol of approximately 2. This stoichiometry is accounted for by the subsequent disproportionation of the ubisemiquinone, which at neutral pH involves the protonated and anionic forms of this species (reaction 4; k_4 varies with pH between 10^4 and 10^7 $M^{-1}s^{-1}$)[13]. Alternatively,

$$\cdot NO + SOD\text{–}Cu^+ \rightarrow NO^- + SOD\text{–}Cu^{++} \quad [2]$$

$$\cdot NO + UQH^- \rightarrow NO^- + UQ\cdot^- \quad [3]$$

$$UQH\cdot + UQ^{\cdot -} \rightarrow UQ + UQH^- \quad [4]$$

reduction of ·NO to NO^- may be accomplished through cytochrome c[14] (reaction 5; $k_5 = 2 \times 10^2 M^{-1}s^{-1}$) and cytochrome oxidase[15] (reaction 6). The contribution of the latter reaction to the reductive decay of ·NO is expected to be small and a distinction should be made between binding and catalysis.

$$\cdot NO + c^{++} \rightarrow NO^- + c^{+++} \quad [5]$$

$$\cdot NO + a_3{}^+ \rightarrow NO^- + aa_3{}^{+++} \quad [6]$$

The binding of ·NO to a partially reduced cytochrome a_3-Cu_B binuclear center occurs at a rate (reaction 7; $k_7 = 4 \times 10^7$ $M^{-1}s^{-1}$) similar to

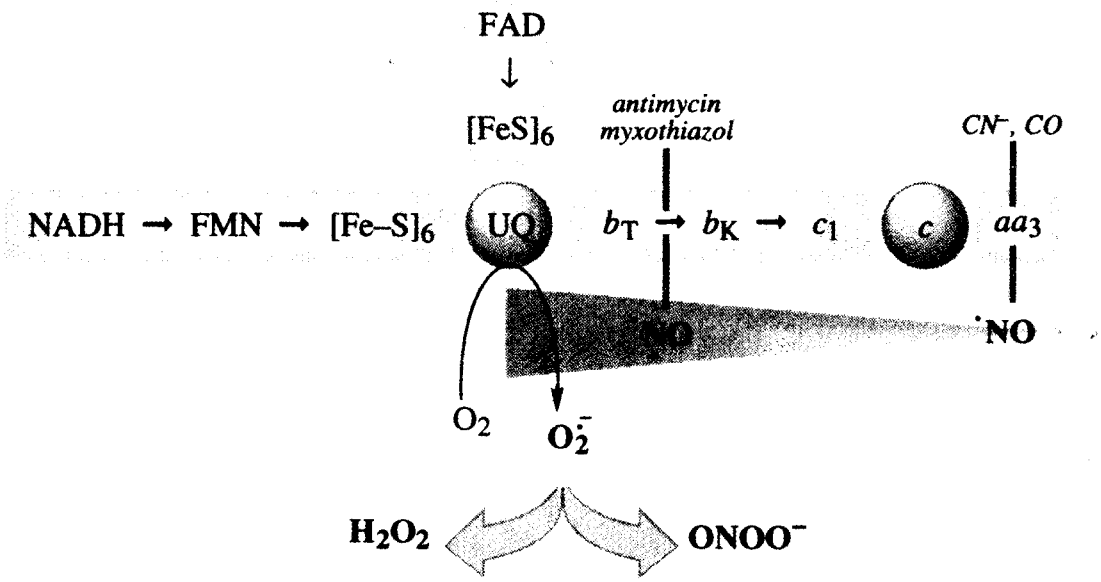

FIGURE 1 Concentration-dependent effects of nitric oxide on the mitochondrial respiratory chain. A simplified scheme of the mitochondrial respiratory chain shows the sites of binding/inhibition or reaction of ·NO (with increasing concentrations). Binding to the bc_1 segment and cytochrome oxidase produces effects similar to antimycin and cyanide, respectively. Reaction with ubiquinol is accomplished at higher concentrations leading to ubisemiquinone formation

that of O_2 ($k = 2 \times 10^8$ $M^{-1}s^{-1}$) and the dissociation of the cytochrome a_3^{2+}-·NO complex proceeds with a first order constant of 0.13 s^{-1} (reaction 8)[16]. This reversible binding to the hemoprotein appears as a major mechanism to regulate mitochondrial functions, but cannot be used for estimation of ·NO steady-state levels. Conversely, the extent of catalysis (·NO → NO^-) (reaction 6) -important for steady-state calculations- is not known and, probably, may account for only a small fraction of ·NO metabolism.

$$\cdot NO + a_3Cu_B^{+} \rightarrow a_3Cu_B^{+} \cdots NO \qquad [7]$$

$$a_3Cu_B^{+} \cdots NO \rightarrow \cdot NO + a_3Cu_B^{+} \qquad [8]$$

The reduction of ·NO by ubiquinol acquires relevance when considering that the ubiquinol content in rat heart mitochondria under normoxic conditions in state 4 is ~1.08×10^{-4} M[17]. This, along with a steady-state concentration of ·NO of ~5×10^{-8} M[18], permits to estimate a rate of utilization of ·NO *via* reaction 3 of ~10^{-8} M s-1 (reaction 9). Moreover, the steady-state levels of ubiquinol in mitochondria may be regulated by ·NO itself upon inhibition of cytochrome oxidase[8,9,19–21] and/or

$$-d[\cdot NO]/dt = k_3[\cdot NO][UQH^-] \qquad [9]$$

binding to the cytochrome bc_1 region[19]. These effects -inhibition of cytochrome oxidase, impairment of electron transfer at the cytochrome bc_1 regions, and oxidation of ubiquinol- require progressively increasing concentrations of this species (Fig. 1). The first two effects are analogous to those exerted by cyanide and antimycin A on the respiratory chain, respectively.

According to the new concept on regulation of cellular respiration mentioned above, the control of mitochondrial functions depends on two variables: ·NO and O_2 tension, each one acting on

concentration range and gradients and intersecting at critical points. Hence, the pathways for ·NO utilization in mitochondria are expected to be modulated by O_2 tensions. It may be proposed that at very low O_2 tensions, mitochondria catalyze ·NO breakdown by two separate mechanisms presumably involving reductive reactions[22]. One of the reductive pathways is sensitive to azide and cyanide and apparently involves reduction of ·NO at the cytochrome oxidase site (reaction 6)[22], although this reaction has not been confirmed with purified cytochrome oxidase[23]. The other reductive pathway may be represented by the interaction of ·NO with ubiquinol as illustrated in reaction 3[12]. The contributions of reaction 6 (and possibly reaction 5) to ·NO reductive decay is relatively low and was calculated to be approximately 4-fold lower than that proceeding through reaction 3[24].

OXIDATIVE DECAY PATHWAYS OF NITRIC OXIDE

In aerobic conditions, the reaction of ·NO with $O_2{\cdot}^-$ would represent the major oxidative decay pathway of ·NO (reaction 10; $k_{10} = 1.9 \times 10^{10}$ $M^{-1}s^{-1}$). Considering mitochondrial steady-state levels of 5×10^{-8} M[18] and 10^{-10} M[25] for ·NO and $O_2{\cdot}^-$, respectively, the actual rate of $ONOO^-$ formation in mitochondria may be estimated as 9.5×10^{-8} M s^{-1} (equation 11)[24]. $ONOO^-$ may also be formed by the reaction of nitroxyl anion with O_2 (reaction 12; $k_{12} = 5.7 \times 10^7$ $M^{-1}s^{-1}$)[26–28].

$$\cdot NO + O_2{\cdot}^- \rightarrow ONOO^- \quad [10]$$

$$+d[ONOO^-]/dt = k_{11}[\cdot NO][O_2{\cdot}^-] \quad [11]$$

$$NO^- + O_2 \rightarrow ONOO^- \quad [12]$$

The chemical reactivity of $ONOO^-$ suggests the formation of a reactive intermediate formed during the isomerization of $ONOO^-$ to NO_3^- (reaction 13); this intermediate is capable of nitration, hydroxylation, and oxidation and may decomposed *via* homolytic O-O bond cleavage to yield HO· and $NO_2{\cdot}$; the recombination of these two radicals can result in nitrate formation.

$$ONOO + H+ \rightarrow ONOOH \rightarrow [HO\cdot + \cdot NO_2] \rightarrow H^+ + NO_3{}^- \quad [13]$$

$ONOO^-$ oxidizes ubiquinol to ubisemiquinone (reaction 14): the reactions involved are first-order in $ONOO^-$ and zero order in ubiquinol[29], in agreement with the rate-limiting formation of a reactive intermediate formed during the isomerization of $ONOO^-$ to NO_3^- (reaction 13). This reaction is expected to proceed freely given (*a*) the reduction potentials of the couples

$$UQH^- + [HO\cdot + \cdot NO_2] \rightarrow UQ^{\cdot -} + \cdot NO_2 + HO^- \quad [14]$$

involved ($E(UQ{\cdot}^-/UQH^-) \sim 0.19$ V; $E(ONOO^-/NO_2{\cdot},HO^-) = +1.4$ V)[30,31] and (*b*) the HO· and $NO_2{\cdot}$-like chemistry of the intermediate: for example, the second order rate constant for the reaction of benzohydroquinone with HO· and $NO_2{\cdot}$ is $>10^9$ $M^{-1}s^{-1}$ and $5 \times 10^8 M^{-1}s^{-1}$, respectively[32].

According to reaction 14, ubiquinol protects against $ONOO^-$-induced protein nitration in mitochondrial membranes. This process requires H abstraction from the tyrosyl residue followed by $NO_2{\cdot}$ addition to the tyrosyl radical (3×10^9 $M^{-1}s^{-1}$)[33]. The mechanistic aspects of this protection cannot be defined precisely, for prevention of nitrotyrosine formation by ubiquinol may involve scavenging of HO·, tyrosyl radical, or $NO_2{\cdot}$ (Fig. 2). All these reactions are thermodynamically feasible when considering the reduction potential of the redox couples involved.

REGULATION OF MITOCHONDRIAL OXYRADICAL PRODUCTION BY NITRIC OXIDE

The reactions of ubiquinol with free radicals are relevant for mitochondrial functions because: (*a*) ubiquinol is a unique component of the electron

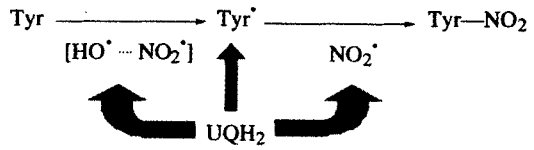

FIGURE 2 Possible targets for ubiquinol during nitrotyrosine formation. The scheme shows the steps leading to nitrotyrosine formation: the reactions of ubiquinol with hydroxyl radical, tyrosyl radical (Tyr·), and NO_2· are thermodynamically feasible

transfer chain, which is efficiently and continuously recycled *via* electrons from complexes I and II, and (*b*) ubisemiquinone, formed in the interactions with free radicals, is a source of oxyradicals in mitochondria. Hence, formation of oxidants by mitochondria may be analyzed in terms of the redox transitions of ubiquinone (reaction 15) associated with, on the one hand, scavenging of free radicals (inherent in the UQH^- → $UQ\cdot^-$ transition) and, on the other hand, formation of $O_2\cdot^-$ (inherent in the $UQ\cdot^-$ → UQ transition).

$$UQH^- \rightarrow UQ^{\cdot -} \rightarrow UQ \qquad [15]$$

Ubisemiquinone autoxidation (reaction 16) is a major mechanism accounting for $O_2\cdot^-$ formation in mitochondria[34,35] as shown by classical experiments using inhibitors of the electron-transfer chain that bound to the O_2 side of the cytochrome *b* segment (*e.g.*, antimycin A and myxothiazol) and that increased the pool of ubisemiquinone.

$$UQ^{\cdot -} + O_2 \rightarrow UQ + UQ\cdot^- \qquad [16]$$

Both ·NO and $ONOO^-$ may contribute to the build up of ubisemiquinone according to the respective reactions (reactions 3 and 14) and, accordingly, to the production of $O_2\cdot^-$ *via* reaction 16:

Nitric oxide. ·NO induces production of H_2O_2 by mitochondria, an effect that requires ubisemiquinone autoxidation accomplished in a sequential manner by, on the one hand, increasing the ubiquinol pool by impairing electron flow at the bc_1 segment[19] and, on the other hand, increasing ubiquinol oxidation by ·NO (reaction 3). Both, ·NO-induced production of H_2O_2 and ·NO utilization are dependent on mitochondrial ubiquinol content. ·NO elicits a biphasic effect on the production of H_2O_2 by mitochondrial membranes[24]: at concentrations below 3 μM, ·NO increased H_2O_2 production, whereas at concentrations above this value, in the range 3–20 μM, ·NO decreased mitochondrial H_2O_2 generation as well as the formation of a DMPO-HO· spin adduct[24]. The decrease of H_2O_2 formation at high ·NO levels is associated with $ONOO^-$ formation. These findings suggest a concentration-dependent regulation by ·NO of the fate of mitochondrial $O_2\cdot^-$: its dismutation to H_2O_2 prevails at low $[\cdot NO]_{SS}$, whereas its conversion to $ONOO^-$ is favored at high $[\cdot NO]_{SS}$[24].

Peroxynitrite. $ONOO^-$ elicits $O_2\cdot^-$ formation by submitochondrial particles[24,29]: a ratio of 0.5 $O_2\cdot^-$ generated *per* $ONOO^-$ added suggests that $ONOO^-$ may exert a regulatory control by supporting a concentration-dependent formation of $O_2\cdot^-$. $ONOO^-$ inhibits complex I and II of the respiratory chain along with the ATP synthase and the aconitase activity and, possibly, activates the mitochondrial permeability transition culminating in apoptosis[36].

The reaction between mitochondrial ubiquinol and ·NO or $ONOO^-$ may involve regulatory and protective aspects:

(*a*) Ubiquinol scavenges free radicals derived from peroxynitrous acid decomposition, thereby protecting mitochondrial proteins against nitration. $ONOO^-$-mediated nitration of mitochondrial proteins is prevented by either increasing the ubiquinone pool upon addition of exogenous ubiquinone or by manipulating the redox status of the pool in favor of ubiquinol[29].

(*b*) The $ONOO^-$-dependent $O_2\cdot^-$ formation by mitochondrial membranes (reaction 14 followed by reaction 16) may be subjected to a

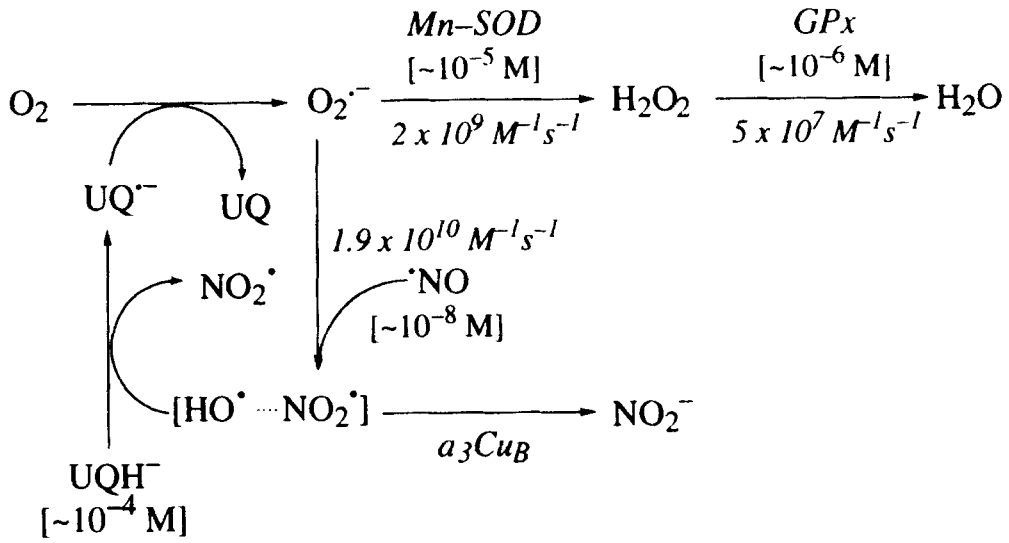

FIGURE 3 Kinetic control on oxygen- and nitrogen-centered radical decay pathways exerted by dismutase and glutathione peroxidase The scheme illustrated the kinetic control ·NO/$ONOO^-$ metabolism exerted by (*a*) enzymes involved in the O_2·- → H_2O_2 → H_2O transition (Mn-superoxide dismutase; Mn-SOD and glutathione peroxidase; GPx) along with the concentrations of these enzymes and rate constants and (*b*) the peroxynitrite reductase activity of cytochrome oxidase

kinetic control imposed by the effective removal of O_2·- by matrix Mn-superoxide dismutase, which yields H_2O_2, in turn reduced to H_2O by mitochondrial glutathione peroxidase (Fig. 3). In addition, the $ONOO^-$ reductase activity of cytochrome oxidase (reaction 17[37], may also exert some kinetic control upon effective removal of $ONOO^-$: the reaction involves

$$ONOO^- + 2e^- + 2H^+ \rightarrow NO_2^- + H_2O \quad [17]$$

mainly a two-electron transfer at the a_3-Cu_B-site of cytochrome oxidase and a minor component (~5%) accounted for by a one-electron transfer process[37].

(*c*) Decay of O_2·- to either H_2O_2 (*via* a Mn-superoxide dismutase-catalyzed reaction; 2.3×10^9 $M^{-1}s^{-1}$) or $ONOO^-$ (*via* its fast reaction with ·NO; reaction 10; $k_{10} = 1.9 \times 10^{10}$ $M^{-1}s^{-1}$) is expected to be a function of the individual mitochondrial concentrations or steady state levels of superoxide dismutase and ·NO.

(*d*) The reactions of ubiquinol with ·NO (reaction 3) and $ONOO^-$ (reaction 14) imply paradoxical effects: on the one hand, the higher utilization of ·NO involved in its reductive decay to nitroxyl anion *via* ubiquinol favor the release of cytochrome oxidase inhibition and, thereby, restores mitochondrial O_2 uptake. On the other hand, the ubiquinol-centered reactions are expected to amplify the formation of $ONOO^-$ (suggested by an enhanced ubisemiquinone autoxidation to yield O_2·- and the fast reaction of the latter with ·NO). As mentioned above, the prevalence of these pathways are partly controlled by the intramitochondrial steady-state level of ·NO: high steady state concentrations of ·NO (as during iNOS induction) are expected to compete efficiently with superoxide dismutase, thereby favoring $ONOO^-$ formation.

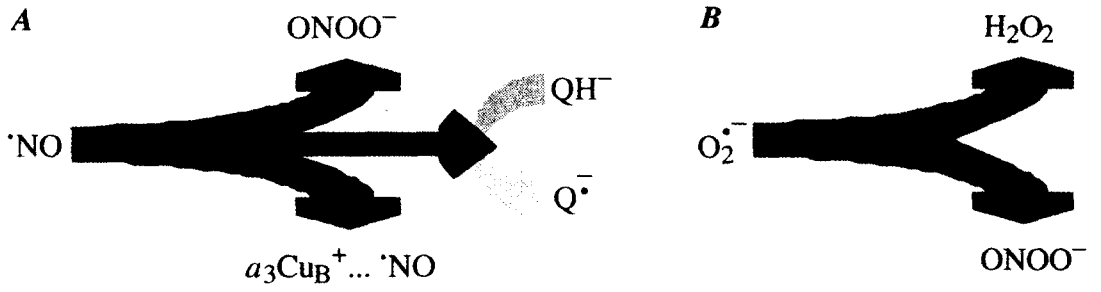

FIGURE 4 Fates of nitric oxide and superoxide in mitochondria. (A) The major metabolic pathways for nitric oxide in mitochondria are its reduction to nitroxyl anion during the ubiquinol redox transition and formation of peroxynitrite, upon its reaction with superoxide. (B) $O_2^{\cdot -}$ is metabolized in mitochondria largely to H_2O_2 and a small portion to $ONOO^-$

STEADY-STATES AND GRADIENTS: MITOCHONDRIAL FATES OF SUPEROXIDE AND NITRIC OXIDE

The rates of ·NO production by mitochondria from different tissues was estimated between 0.2-and 7.0 × 10^{-5} Ms^{-1}(mouse thymus- and rat liver mitochondria showing the lowest and highest values, respectively)[7,38,39]. The rates of superoxide formation by mitochondria (submitochondrial particles) is in the 10^{-6} Ms^{-1} range. Although the fast reaction of ·NO with muscle cytosolic myoglobin would certainly exert a profound influence on the mitochondrial and cellular steady-state levels of ·NO in this tissue, it was not considered for calculations of mitochondria from other tissues. The steady-state concentration of superoxide in mitochondria has been calculated as 10^{-10} M[25] and that of ·NO as 10^{-8}M[18]. In rat liver mitochondria under normoxic conditions, ·NO is largely metabolized through the oxidation of ubiquinol and formation of $ONOO^-$ and, secondarily through reduction by cytochrome oxidase and/or cytochrome *c* (Fig. 4A). H_2O_2 formation accounts for most of the $O_2^{\cdot -}$ formed in mitochondria and a small fraction is metabolized to $ONOO^-$ (Fig. 4B)[24].

Table I summarizes the reactions considered to be the main sinks of ·NO[16] in mitochondria, as well the overall rate consumption of ·NO by intact mitochondria. The binding reactions to hemoproteins are not included, because these reactions do not represent a true consumption of ·NO [40]. A ·NO steady-state level of either 3 × 10^{-8}M (that implies ~33% inhibition of cytochrome oxidase[41]) or 3 × 10^{-7} M (a concentration in the membrane phase) is assumed in these calculations. A concentration of ·NO of 6 × 10^{-8} M was calculated to be required to raise the K_M for O_2 to 1 × 10^{-5} M by applying a Michaelis scheme to data from cultured cells producing ·NO at a concentration suitable to depress respiration upon cytochrome oxidase inhibition[42].

Two major groups of reactions can be distinguished in Table I: those whose rate proceeds or can be calculated in the 10^{-7}-10^{-9} M s^{-1} range and those which occur at much slower rates, in the range 10^{-12}-10^{-14} M s^{-1}.

The former group includes (*a*) the reaction with ubiquinol, that constitutes the main sink of ·NO (v_3 in mitochondria may be calculated as 1.9 × 10^{-7} Ms^{-1}). Concentrations used were those of ubiquinol and ·NO in the lipid phase; (*b*) The flux of the reaction of ·NO with $O_2^{\cdot -}$, forming $ONOO^-$, was estimated at 5.7 × 10^{-8} Ms^{-1} representing also an important sink of ·NO. (*c*) The reduction of ·NO by cytochrome *c* appears to be a minor pathway within this group.

TABLE I Main catabolic pathways of ·NO in mitochondria

Reaction	*[Species]$_{SS}$ (M)*		*k*	*Rate (Ms^{-1})*
$\cdot NO + UQH^- \rightarrow NO^- + UQ^{\cdot-}$	$[\cdot NO]_{SS} = 3 \times 10^{-7}$[a]	$[UQH^-]_{SS} = 1.6 \times 10^{-3}$[a]	$2.3 \times 10^3\ M^{-1}s^{-1}$	1.9×10^{-7}[b]
$\cdot NO + O_2^{\cdot-} \rightarrow ONOO^-$	$[\cdot NO]_{SS} = 3 \times 10^{-8}$	$[O_2^{\cdot-}]_{SS} = 1.0 \times 10^{-10}$	$1.9 \times 10^{10}\ M^{-1}s^{-1}$	5.7×10^{-8}
$\cdot NO + c^{2+} \rightarrow NO^+ + c^{3+}$	$[\cdot NO]_{SS} = 3 \times 10^{-8}$	$[c^{2+}]_{SS} = 2.0 \times 10^{-4}$	$2.0 \times 10^2\ M^{-1}s^{-1}$	1.2×10^{-9}
$2\cdot NO + O_2 \rightarrow 2NO_2\cdot$	$[\cdot NO]_{SS} = 3 \times 10^{-7}$[a]	$[O_2]_{SS} = 3.0 \times 10^{-5}$[a]	$6.3 \times 10^6\ M^{-2}s^{-1}$	3.4×10^{-12}[b]
$2\cdot NO + O_2 \rightarrow 2NO_2\cdot$	$[\cdot NO]_{SS} = 3 \times 10^{-8}$	$[O_2]_{SS} = 1.0 \times 10^{-5}$	$6.3 \times 10^6\ M^{-2}s^{-1}$	5.7×10^{-14}
$\cdot NO + a_3^{+} \rightarrow NO^- + a_3^{2+}$	$[\cdot NO]_{SS} = 3 \times 10^{-8}$	$[a_3^{2+}\text{-}\cdot NO]_{SS} = 6.0 \times 10^{-5}$	$<0.13\ s^{-1}$	$<2.3 \times 10^{-13}$[c]
Mitochondrial production				2.3×10^{-5}
Mitochondrial consumption			$6\ s^{-1}$	

a. Concentrations referred to membrane phase.
b. Rates referred to total mitochondrial volume were obtained by dividing the rate in the membrane phase by a factor of 5, which accounts for the ratio between mitochondria and membrane volume.
c. The rate constant for the reduction of ·NO by cytochrome oxidase is not known; however, this rate constant should be lower than the dissociation constant ($0.13\ s^{-1}$) of the complex a_3^{2+}-·NO[16].

The latter group includes the reaction of ·NO with cytochrome oxidase and with O_2 (calculated as a function of $[\cdot NO]_{SS}$ in the matrix and in the membrane phase), which represent only small sinks of ·NO.

From the half-life of ·NO (1.8 min)[24] in the presence of 1 mg mitochondrial protein/ml, a pseudo-first order rate constant of 6 s^{-1}may be calculated for the consumption of ·NO by mitochondria. From the sum of the reactions in Table I, a similar pseudo-first order rate constant (8.2 s^{-1}) may be estimated: this indicates that in fact the reactions listed in Table I cover the main mitochondrial processes that consume ·NO. A striking feature implied by these calculations is that the sum of all the reactions consuming ·NO is only a small fraction (~1%) of the estimated flux of ·NO production (2.3×10^{-5} Ms^{-1} or 1.4 nmol/min/mg of protein[38]). Assuming a rate of production of ·NO of 2.3×10^{-5} Ms^{-1} and a pseudo-first order rate constant of 6 s^{-1} for the consumption ·NO a steady-state for ·NO of 3.8×10^{-6} M may be obtained. Such steady-state is unreasonably high and would imply an inhibition of cytochrome oxidase of ≈95%. Hence, it may be surmised that either other processes consuming ·NO need be considered or the steady-state concentration of ·NO is higher than that assumed or that the rate of ·NO release by mitochondria is overestimated.

The data listed in Table I does not distinguish between an intramitochondrial source of ·NO and ·NO diffusing into mitochondria from a cellular source. Regardless of the origin of this second messenger, it is clear that it regulates mitochondrial functions and mitochondrial oxyradical production, exerting changes that contribute to cell-death pathways.

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