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# Nitric oxide metabolism in heart mitochondria

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**ABSTRACT:** Normal cardiac function is accomplished through a continuous energy supply provided by mitochondria. Heart mitochondria are the major source of reactive oxygen and nitrogen species: superoxide anion ($O_2^-$) and nitric oxide (NO). NO production by mitochondrial NOS (mtNOS) is modified by metabolic state and shows an exponential dependence on $\Delta\psi$. The interaction between mtNOS and complexes I and IV might be a mechanism involved in the regulation of mitochondrial NO production. NO exerts a high affinity, reversible and physiological inhibition of cytochrome *c* oxidase activity. A second effect of NO on the respiratory chain is accomplished through its interaction with ubiquinol-cytochrome *c* oxidoreductase. The ability of mtNOS to regulate mitochondrial $O_2$ uptake and $O_2^-$ and $H_2O_2$ productions through the interaction of NO with the respiratory chain is named mtNOS functional activity. Together, heart mtNOS allows NO to optimize the balance between cardiac energy production and utilization, and to regulate the steady-state concentrations of other oxygen and nitrogen species.

Normal cardiac function is accomplished through a continuous energy supply provided by mitochondria, which produce 95% of total cellular ATP by oxidative phosphorylation. Mitochondria play a central role in various biological processes providing the cell with both the energy and the signals involved in the genetic expression and metabolic regulation (Darley-Usmar, 2004). Indeed, heart mitochondria are the major source of reactive oxygen and nitrogen species. These species are mainly derived from two primary free radicals: superoxide anion ($O_2^-$) and nitric oxide (NO). Superoxide anion is the precursor of hydrogen peroxide ($H_2O_2$) and is generated within mitochondria through the autoxidation of the intermediate semiquinones ($UQH^•$ and $FMNH^•$) of the redox pairs ubiquinol/ubiquinone at complex III, and FMNH2/FMN component of the NADH dehydrogenase (Boveris and Cadenas, 2000).

In addition, mitochondria produce NO in a reaction catalyzed by mitochondrial nitric oxide synthase (mtNOS) (Zaobornyj and Ghafourifar, 2012). This isoform is located in the inner mitochondrial membrane and it was identified as the α-nNOS variant with post-translational modifications (Elfering *et al.,* 2002). Central roles have been postulated for mtNOS in chronic hypoxia, ischemia-reperfusion, cell signaling, aging, dystrophin deficiency, inflammation, and cancer. The mtNOS activity has been found up-regulated in cold acclimation (Peralta *et al.,* 2003). Treatment with enalapril produced an increase in the production of NO by heart mitochondrial membranes (Boveris *et al.,* 2003). Lately, a receptor for angiotensin was found located in inner mitochondrial membrane and it was suggested that the renine-angiotensin system directly regulates mitochondrial NO production (Abadir *et al.,* 2011). In addition, heart

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mtNOS activity was shown to be increased in experimental endotoxemia (Alvarez and Boveris, 2004). Therefore, a variety of physiological, pathological, and pharmacological situations can cause changes in heart mtNOS activity or expression.

Such a spatially restricted localization of NO production in mitochondria elicits a regulation of NOS activity by the local environment within these *organelles,* established by the concentration of $O_2$, $Ca^{2+}$, L-arginine, membrane potential or redox state (reduced glutathione (GSH)/oxidized glutathione (GSGG) balance). Interestingly, mitochondrial NO production is influenced by metabolic state (Valdez *et al.,* 2006). During the transition from resting (state 4) to active (state 3) respiration, heart mitochondrial NO release decreases about 60%. In addition, heart mitochondrial NO production shows a strong and exponential dependence on the mitochondrial transmembrane potential ($\Delta\psi$). This dependence is more pronounced in the physiological range of $\Delta\psi$ where small changes in the $\Delta\psi$ produce noticeable variations of mitochondrial NO release. To date, several studies have shown that abolishing $\Delta\psi$ inhibits mtNOS activity, indicating a tight regulatory interplay between mitochondrial NO production and $\Delta\psi$ (Dedkova and Blatter, 2009; Kanai *et al.,* 2001).

Some authors have reported a structural and functional interaction among complexes I, IV and mtNOS (Franco *et al.,* 2006; Persichini *et al.,* 2005). A physical proximity of mtNOS with the C-terminal peptide of the Va subunit of cytochrome *c* oxidase has been shown by electron microscopic immunolocalization and co-immunoprecipitation studies. Other report showed that not only complex IV but also complex I proteins immunoprecipitate with nNOS, which indicated a direct molecular interaction between mtNOS and complexes I and IV. This association might be one of the mechanisms involved in the regulation of mitochondrial NO production. In our laboratory, heart submitochondrial particles (SMP) produced about 1.0 nmol $NO.min^{-1}.mg$ $protein^{-1}$, in conditions of reverse electron flow and without the addition of the classical NOS electron donor NADPH. This observation suggested that NO production can be supported by electrons derived from the low isopotential components of the respiratory chain. Moreover, the complex I inhibitor rotenone totally inhibited NO production supported by reverse electron transfer but did not reduce the activity of recombinant nNOS, indicating that the effect of rotenone on NO production by SMP is due to an electron flow blockage and not to a direct action on NOS structure.

The presence of NOS in cardiac mitochondria provides a mechanism for the fine regulation of the respiratory complexes and of the enzymes of the citric acid cycle. It is well known that NO activates soluble guanylate cyclase (sGC) and that this activation leads to the production of 3',5'-cyclic guanosine monophosphate (cGMP), which in turn acts as a second messenger (Layland *et al.,* 2002). Nevertheless, certain key functions of NO are exerted through cGMP-independent pathways. At physiological sub-micromolar concentrations, most distinct effects of NO on mitochondria are conveyed to the respiratory chain and thereby on energy metabolism (Moncada and Erusalimsky, 2002).

First, NO exerts a high affinity, reversible and physiological inhibition of cytochrome *c* oxidase activity (Brown and Cooper, 1994; Cleeter *et al.,* 1994). It is worth noting that this effect occurs through a direct competition between NO and $O_2$ for the two-electron-reduced $Cu_B$/heme $a_3$ center of the enzyme. Antunes and co-workers explained the reversible inhibition of COX by NO using a mathematical model based in experimental data and described different effects of NO in the mitochondrial metabolic states (2004; 2007). A second important effect of NO on mitochondrial respiratory chain is accomplished through a recently described interaction of NO with the respiratory complex III, ubiquinol-cytochrome c oxidoreductase (Iglesias *et al.,* 2015). The inhibition of the respiratory chain between cytochromes *b* and *c* by NO was assessed using SMP and two different NO donors: S-nitrosoglutathione and spermine-NONOate. The activity of succinate-cytochrome *c* reductase (complex II-III) was inhibited by about 50% in the presence of the NO donors, at ~1.25 μM NO, whereas the activity of succinate-Q reductase (complex II) resulted unaffected. These data indicated that NO specifically inhibits complex III. Interestingly, complex II-III activity was also decreased (36%) when SMP were incubated with mtNOS substrates and cofactors, suggesting that the inhibition is also produced by endogenous NO. In addition, this study showed hyperbolic increases in $O_2^-$ and $H_2O_2$ production rates with a maximal effect at 500 μM GSNO. Moreover, $H_2O_2$ production by heart coupled mitochondria was increased by 75% when mitochondria were exposed to the NO donors. Finally, an EPR signal al $g$=1.99 that would denote the formation of a stable semiquinone ($UQH^{\bullet}$), was detected when SMP were incubated in the presence of succinate. This EPR signal was increased not only by the classic complex III inhibitor antimycin but also by GSNO and SPER-NO. These results indicate that NO interacts with ubiquinone-cytochrome *b* area producing antimycin-like effects.

The ability of mtNOS to regulate mitochondrial $O_2$ uptake and $H_2O_2$ production, through the interaction of NO with the respiratory chain, has been named mtNOS functional activity (Valdez *et al.,* 2005). This activity is determined by the difference in the rates of $O_2$ uptake or $H_2O_2$ production in isolated mitochondria in two conditions. Under the condition when NO steady state levels are the highest, i.e. in the presence of sufficient L-arginine and SOD, active $O_2$ consumption is impaired and $H_2O_2$

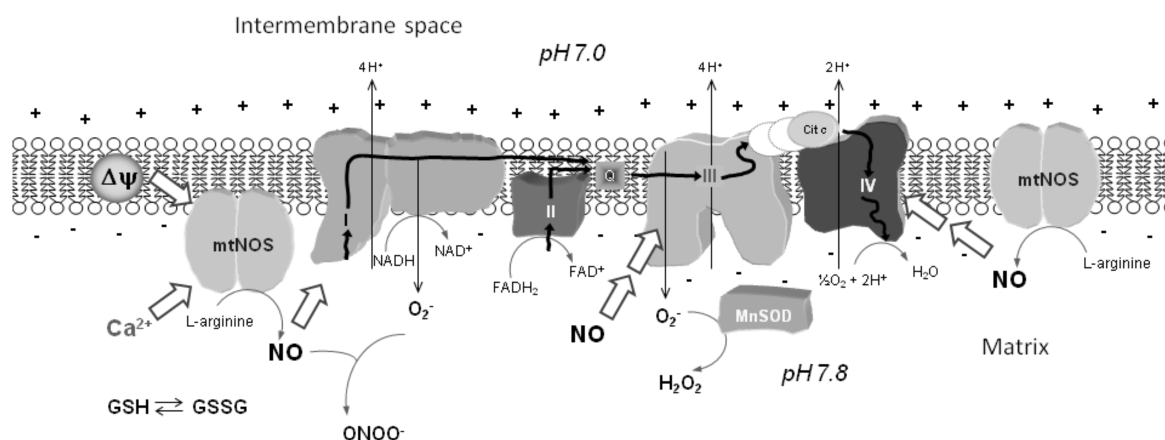


FIGURE 1. Reciprocal regulation of mtNOS activity and mitochondrial function. Localization of NOS within mitochondria provides a distinct specific regulation of mitochondrial NO production by intramitochondrial $O_2$, $Ca^{2+}$, Δψ, L-arginine, or redox state (GSH/GSGG balance). Nitric oxide produced by mtNOS can readily react with mitochondrial targets such as respiratory complexes I, III or IV. The $O_2^-$ is formed at the respiratory chain and undergoes a very fast reaction with NO to form $ONOO^-$ or it is catabolized to form $H_2O_2$.

production is enhanced. On the contrary, when NO steady state levels are the lowest, active $O_2$ consumption is increased and $H_2O_2$ production is decreased. Such situation is achieved in the absence of NOS substrates or cofactors, or in the presence of a NOS inhibitor or a NOS scavenger, e.g. oxyhemoglobin. Thus, changes in mtNOS functional activity reveal modifications of NO production by mtNOS.

Other effects of NO in cell signaling are conveyed via nitrosation of proteins (Stamler *et al.,* 2001). Importantly, complex I is a target for reversible inhibition by S-nitrosation of critical thiol residues and this reaction appears to be crucial in cardioprotective procedures such as ischemic preconditioning (Burwell *et al.,* 2006). Nitric oxide also reacts with $O_2^-$ that is formed by the mitochondrial respiratory chain during normal or pathological metabolism (Koppenol *et al.,* 1992). The product is peroxynitrite ($ONOO^-$), a non-radical species capable of oxidizing and nitrating biomolecules and damaging irreversibly those targets (Radi *et al.,* 2002). Peroxynitrite may hinder mitochondrial functions and cause cell death. Diverse factors participate in the switch from reversible inhibition of cellular respiration by NO to the pathological inhibition of mitochondrial function by $ONOO^-$. Through its interactions with components of the electron transfer chain, NO functions not only as a physiological inhibitor of cell respiration and ATP production, but it also prevents or activates mitochondrial permeability transition opening, participates in $Ca^{2+}$ homeostasis (Dedkova and Blatter, 2009), enhances the generation of reactive species (Iglesias *et al.,* 2015), and thereby triggers various mechanisms underlying cellular survival or death (Moncada and Erusalimsky, 2002).

In conclusion, NO is a central molecule involved in the modulation of heart function, and in key processes of cardiac metabolism. Fig. 1 depicts the reciprocal regulation of mtNOS activity and mitochondrial function. The effect of Δψ on mtNOS activity allows the enzyme to respond to changes in cardiomyocyte energy homeostasis. Furthermore, heart mtNOS is regulated by a variety of physiological, pathological, and pharmacological situations. At the same time, NO generated within mitochondria has focused effects on mitochondrial function. Together, heart mtNOS allows NO to optimize the balance between cardiac energy production and utilization, and to regulate processes such as oxygen and nitrogen free radical production and cell survival.

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## References

Abadir PM, Foster DB, Crow M, Cooke CA, Rucker JJ, Jain A, Smith BJ, Burks TN, Cohn RD, Fedarko NS, Carey RM, O'Rourke B, Walston JD (2011). Identification and characterization of a functional mitochondrial angiotensin system. *Proceedings of the National Academy of Sciences (USA)* **108:** 14849-14854.

Alvarez S, Boveris A (2004). Mitochondrial nitric oxide metabolism in rat muscle during endotoxemia. *Free Radical Biology and Medicine* **3:** 1472-1478.

Antunes F, Boveris A, Cadenas E (2004). On the mechanism and biology of cytochrome oxidase inhibition by nitric oxide. *Proceedings of the National Academy of Sciences (USA)* **101:** 16774-16779.

Antunes F, Boveris A, Cadenas E (2007). On the biologic role of the reaction of NO with oxidized cytochrome *c* oxidase. *Antioxidant and Redox Signaling* **9:** 1569-1579.

Boveris A, D'Amico G, Lores-Arnaiz S, Costa LE (2003). Enalapril increases mitochondrial nitric oxide synthase activity in heart and liver. *Antioxidant and Redox Signaling* **5:** 691-697.

Boveris A, Cadenas E (2000). Mitochondrial production of hydrogen peroxide regulation by nitric oxide and the role of ubisemiquinone. *IUBMB Life* **50:** 245-50.

Brown GC, Cooper CE (1994). Nanomolar concentrations of nitric oxide reversibly inhibit synaptosomal respiration by competing with oxygen at cytochrome oxidase. *FEBS Letters* **356:** 295-298.

Burwell LS, Nadtochiy SM, Tompkins AJ, Young S, Brookes PS (2006). Direct evidence for S-nitrosation of mitochondrial complex I. *Biochemical Journal* **394:** 627-634.

Cleeter MW, Cooper JM, Darley-Usmar VM, Moncada S, Schapira AH (1994). Reversible inhibition of cytochrome c oxidase, the terminal enzyme of the mitochondrial respiratory chain, by nitric oxide. Implications for neurodegenerative diseases. *FEBS Letters* **345:** 50-54.

Darley-Usmar V (2004) The powerhouse takes control of the cell; the role of mitochondria in signal transduction. *Free Radical Biology and Medicine* **37:** 753-754.

Dedkova EN, Blatter LA (2009). Characteristics and function of cardiac mitochondrial nitric oxide synthase. *Journal of Physiology* **587:** 851-872.

Elfering SL, Sarkela TM, Giulivi C (2002). Biochemistry of mitochondrial nitric-oxide synthase. *Journal of Biological Chemistry* **277:** 38079-38086.

Franco MC, Arciuch VG, Peralta JG, Galli S, Levisman D, Lopez LM, Romorini L, Poderoso JJ,Carreras MC (2006). Hypothyroid phenotype is contributed by mitochondrial complex I inactivation due to translocated neuronal nitric-oxide synthase. *Journal of Biological Chemistry* **281:** 4779-4786.

Iglesias DE, Bombicino SS, Valdez LB, Boveris A (2015). Nitric oxide interacts with mitochondrial complex III producing antimycin-like effects. *Free Radical Biology and Medicine* **89:** 602-613.

Kanai AJ, Pearce LL, Clemens PR, Birder LA, Vanbibber MM, Choi SY, De Groat WC, Peterson J (2001). Identification of a neuronal nitric oxide synthase in isolated cardiac mitochondria using electrochemical detection. *Proceedings of the National Academy of Sciences (USA)* **98:** 14126-14131.

Koppenol WH, Moreno JJ, Pryor WA, Ischiropoulos H, Beckman JS (1992). Peroxynitrite, a cloaked oxidant formed by nitric oxide and superoxide. *Chemical Research in Toxicology* **5:** 834-842.

Layland J, Li JM, Shah AM (2002). Role of cyclic GMP-dependent protein kinase in the contractile response to exogenous nitric oxide in rat cardiac myocytes. *Journal of Physiology* **540:** 457-467.

Moncada S, Erusalimsky JD (2002). Does nitric oxide modulate mitochondrial energy generation and apoptosis? *Nature Reviews Molecular Cell Biology* **3:** 214-220.

Peralta JG, Finocchietto PV, Converso D, Schopfer F, Carreras MC,Poderoso JJ (2003). Modulation of mitochondrial nitric oxide synthase and energy expenditure in rats during cold acclimation. *American Journal of Physiology - Heart Circulatory Physiology* **284:** H2375-2383.

Persichini T, Mazzone V, Polticelli F, Moreno S, Venturini G, Clementi E, Colasanti M (2005). Mitochondrial type I nitric oxide synthase physically interacts with cytochrome *c* oxidase. *Neuroscience Letters* **384:** 254-259.

Radi R, Cassina A, Hodara R, Quijano C, Castro L (2002). Peroxynitrite reactions and formation in mitochondria. *Free Radical Biology and Medicine* **33:** 1451-1464.

Stamler JS, Lamas S, Fang FC (2001). Nitrosylation. the prototypic redox-based signaling mechanism. *Cell* **106:** 675-683.

Valdez LB, Zaobornyj T, Boveris A (2005). Functional activity of mitochondrial nitric oxide synthase. *Methods in Enzymology* **396:** 444-455.

Valdez LB, Zaobornyj T, Boveris A (2006). Mitochondrial metabolic states and membrane potential modulate mtNOS activity. *Biochimica et Biophysica Acta* **1757:** 166-172.

Zaobornyj T, Ghafourifar P (2012). Strategic localization of heart mitochondrial NOS: a review of the evidence. *American Journal of Physiology - Heart Circulatory Physiology* **303:** 1283-1293.