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Forum Editorial

Mitochondria and Nitric Oxide

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EACH YEAR, RESEARCH SCIENTISTS provide new and exciting insights into the roles that reactive oxygen and nitrogen species (RNOS) play in normal and abnormal cell functions. The number of published studies on oxygen, and more recently on nitrogen, radicals-mediated chemistry has risen exponentially since the early 1980s, when these radicals were considered, well, radical. Initially, interest in nitric oxide (NO)-mediated regulation was focused on soluble guanylate cyclase, a hemoprotein involved in cyclic GMP-mediated cell signaling and a clear target for NO attack. It has become clear over the last decade, however, that many other hemoproteins, thiols, and other reactive oxygen species (ROS) can enter into chemical reactions with NO. Mitochondria possess multiple targets for NO action, including hemoproteins such as cytochrome oxidase, protein, and lipid thiols, and are a cellular source of ROS such as superoxide anion and hydrogen peroxide. It is not surprising, then, that NO interaction with mitochondria would have significance for cell function.

As in many biochemical reactions, location matters and the discovery of a functional mitochondrial NO synthase (mtNOS) in 1997 (5) has spotlighted a highly localized source of NO in the mitochondrial inner membrane. A unique feedback regulation of mitochondrial respiration has emerged that involves mtNOS and NO generation. This mechanism may serve as an adaptive response to numerous cellular metabolic challenges, such as hypoxia. What makes mtNOS a very peculiar member of the NOS family and predicts an apparently contradictory role for NO, is that the NO born within mitochondria is like Odysseus born in Troy! The basal levels of NO produced by the endogenous basal mtNOS activity serve as one of the main regulators of mitochondrial and, thus, cellular bioenergetics. Basal mtNOS activity keeps mitochondrial respiration lowered (5), and thus regulates mitochondrial transmembrane potential ($\Delta\Psi$) (5), transmembrane pH gradient (ΔpH), and Ca^{2+} homeostasis (6). These basal levels of NO also play an antioxidant role by reacting with potentially harmful ROS, disposing of them on mitochondrial reducing defenses such as reduced glutathione (GSH). However, overstimulation of mtNOS by loading mitochondria with Ca^{2+} overproduces NO in the heart of the cellular source of ROS, unloads the Trojan Horse, and results in the generation of RNOS that mitochondrial reducing barriers cannot neutralize (7). The protective walls are broken, and thus oxidative stress and ultimately cell death occur.

This special edition of *Antioxidants & Redox Signaling* typifies the continuing investigation that expands our knowledge on oxygen and nitrogen radicals in a multitude of normal cell functions and disease states. Neurodegenerative diseases including Parkinson's disease (PD), the polyglutamine repeat diseases like Huntington's disease, amyotrophic lateral sclerosis, Alzheimer's disease (AD), neuroinflammatory diseases like multiple sclerosis (MS), as well as more acute neuropathological states such as stroke, are frequently characterized as diseases of oxidative stress. Some researchers would argue that the redox imbalance is a primary causative element, and signs of oxidative stress are visible as early and definable pathological features of several diseases. In both PD and AD, neurons that are critical to the disease locus, the dopaminergic striatal neurons for PD and the hippocampal neurons for AD, demonstrate markers of protein, lipid, and nucleic acid oxidation prior to the appearance of the most dramatic pathological features such as amyloid deposits, neurofibrillary tangles, or α-synuclein aggregates within Lewy bodies. As ROS have long been known as polymerizing agents, it is not surprising that aggregation of proteins might involve oxidative processes. What may be surprising, however, is that the mitochondria that power a cell's survival also provide the lion's share of the reactive species that influence disease and, ultimately, lead to cell death.

This forum issue is adorned by several exciting articles. The group of Boveris report on the existence of mtNOS in rat renal cortex and the effect of enalapril in increasing the mtNOS protein expression and activity (1). Sharma and Ebadi (10) describe the attenuation of 3-morpholinosydnonimine-induced oxidative stress in dopaminergic neurons by induction of the metallothionein gene. Shilo and Tirosh (11) focus on the role

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of mitochondrial oxidative stress in selenite-induced activation of a caspase-independent cell death in Jurkat T cells and J774.2. Lemeshko's lab introduces otobaphenol, a lignan extract of *Virola Aff. Pavonis* leaves, and demonstrates the antioxidant properties of the phenol by showing that it inhibits the Fe^{3+}-ascorbate-induced lipoperoxidation of rat liver mitochondria and that it delays the mitochondrial permeability transition induced by loading mitochondria with Ca^{2+} (9).

In this issue, Ghafourifar and Colton discuss the suborganelle compartmentalized nitrosation and nitration in mitochondria (4); Seung-Jae Lee (8) elegantly discusses the interplay between oxidation and mitochondrial function and how these affect aggregation of α-synuclein; Mike Murphy (2) illuminatingly explains the mitochondrial thiol metabolism and how NO and its metabolites interact with mitochondrial thiols; Manuchair Ebadi (3) skillfully discusses the reactive nitrogen species and mitochondrial dysfunctioning in PD and how selegiline, a monoamine oxidase-B inhibitor, inhibits apoptosis and prevents loss of mitochondrial transmembrane potential in PD; and David Wink (12) persuasively describes the chemistry of nitrosylation and the interactions of NO and other nitrogen oxides with hemoproteins and metal-oxo species, such as peroxidases and monooxygenases.

The source of the oxidative stress in diseases such as AD, PD, or MS remains a central question, garnering great interest and debate. This issue of *Antioxidants & Redox Signaling* presents the emerging view that oxidative stress cannot be considered without also considering NO. Complex RNOS, formed initially by the interaction of NO and ROS, can generate oxidative chemistry. The best known and most controversial of the RNOS-derived oxidants is peroxynitrite. However, it is important to realize that other RNOS species such as HNO or NO_2 as delineated by Thomas *et al.* (12) in this issue can serve as cellular oxidants capable of forming nitrotyrosines, one of the most common footprints of oxidative stress in diseases. In this case, the interaction of RNOS with metal-oxo complexes such as those found in peroxidases are central to the oxidant stress.

How mitochondria regulate the mtNOS activity to orchestrate the antioxidative versus oxidative feature of mtNOS remains to be further elucidated. Nature broadly uses feedback systems to regulate many events; thus, many biological processes rely heavily on feedback mechanisms. As mtNOS regulates the electron transport chain of mitochondria through antagonizing O_2 at the level of cytochrome oxidase, it seems very logical that the electrons of the mitochondrial respiratory chain counterregulate the mtNOS activity. How this feedback system is constructed is not yet revealed. mtNOS, like other NOS, requires reducing equivalents to convert L-arginine to NO. For other presently known NOS isozymes, NADPH serves as the electron donor; however, mtNOS is associated with the mitochondrial inner membrane, a high trafficking highway of electrons. Our (Ghafourifar and Colton) yet unpublished observations indicate that a portion of electrons passing through the mitochondrial electron chain at a site near respiratory chain complex I are diverted to mtNOS. It seems that, as opposed to other presently known members, the youngest member of the NOS family may actually not be NADPH-dependent. These novelties of mtNOS offer another classic example of nature's wonders and provide a continuing power to surprise.

ABBREVIATIONS

AD, Alzheimer's disease; MS, multiple sclerosis; mtNOS, mitochondrial nitric oxide synthase; NO, nitric oxide; NOS, nitric oxide synthase; PD, Parkinson's disease; RNOS, reactive nitrogen and oxygen species; ROS, reactive oxygen species.

REFERENCES

1. Boveris A, Valdez LB, Alvarez S, Zaobornyj T, Boveris AD, and Navarro A. Kidney mitochondrial nitric oxide synthase. *Antioxid Redox Signal* 5: 265–271, 2003.
2. Costa NJ, Dahm CC, Hurrell F, Taylor ER, and Murphy MP. Interactions of mitochondrial thiols with nitric oxide. *Antioxid Redox Signal* 5: 291–305, 2003.
3. Ebadi M and Sharma SK. Peroxynitrite and mitochondrial dysfunction in the pathogenesis of Parkinson's disease. *Antioxid Redox Signal* 5: 319–335, 2003.
4. Ghafourifar P and Colton CA. Compartmentalized nitrosation and nitration in mitochondria. *Antioxid Redox Signal* 5: 349–354, 2003.
5. Ghafourifar P and Richter C. Nitric oxide synthase activity in mitochondria. *FEBS Lett* 418: 291–296, 1997.
6. Ghafourifar P and Richter C. Mitochondrial nitric oxide synthase regulates mitochondrial matrix pH. *Biol Chem* 380: 1025–1028, 1999.
7. Ghafourifar P, Schenk O, Klein SD, and Richter C. Mitochondrial nitric oxide synthase stimulation causes cytochrome *c* release from isolated mitochondria. Evidence for intramitochondrial peroxynitrite formation. *J Biol Chem* 274: 31185–31188, 1999.
8. Lee SJ. α-Synuclein aggregation: a link between mitochondrial defects and Parkinson's disease? *Antioxid Redox Signal* 5: 337–348, 2003.
9. Lemeshko VV, Lopez LF, Solano S, and Torres R. The natural antioxidant otobaphenol delays the permeability transition of mitochondria and induces their aggregation. *Antioxid Redox Signal* 5: 281–290, 2003.
10. Sharma SK and Ebadi M. Metallothionein attenuates 3-morpholinosydnonimine (SIN-1)-induced oxidative stress in dopaminergic neurons. *Antioxid Redox Signal* 5: 251–264, 2003.
11. Shilo S and Tirosh O. Selenite activates caspase-independent necrotic cell death in Jurkat T cells and J774.2 macrophages by affecting mitochondrial oxidant generation. *Antioxid Redox Signal* 5: 273–279, 2003.
12. Thomas DD, Miranda KM, Colton CA, Citrin D, Espey MG, and Wink DA. Heme proteins and nitric oxide (NO): the neglected, eloquent chemistry in NO redox signaling and regulation. *Antioxid Redox Signal* 5: 307–317, 2003.

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1. Lissbeth Leon, Jean-François Jeannin, Ali Bettaieb. 2008. Post-translational modifications induced by nitric oxide (NO): Implication in cancer cells apoptosis#. *Nitric Oxide* **19**:2, 77-83. [CrossRef]
2. Tienush Rassaf, Malte KelmNitrite and nitrosospecies in blood and tissue 269-288. [CrossRef]