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## Hypoxia exacerbates macrophage mitochondrial damage in endotoxic shock

Ana Navarro<sup>1</sup> and Alberto Boveris<sup>2</sup>

<sup>1</sup>Department of Biochemistry and Molecular Biology, Faculty of Medicine, University of Cádiz, Cádiz, Spain; and <sup>2</sup>Laboratory of Free Radical Biology, School of Pharmacy and Biochemistry, University of Buenos Aires, Buenos Aires, Argentina

SEPSIS AND SEPTIC SHOCK are major causes of death after trauma and a persistent problem in surgical patients. The prevalent hypothesis regarding the mechanism of sepsis and septic shock is that the syndrome is caused by an excessive defensive and inflammatory response with massive increases of NO and inflammatory cytokines in body fluids, systemic damage to vascular endothelium, and impaired tissue and whole body respiration despite adequate O<sub>2</sub> supply. Human muscle biopsies from shock patients show, in correlation with the clinical severity of the case, NO overproduction, glutathione depletion, and mitochondrial respiratory dysfunction with decreased ATP levels and O<sub>2</sub> consumption, the latter especially with NAD-linked and complex I-dependent substrates (3, 4, 16). The selective mitochondrial damage associated with sepsis and septic shock has been widely observed and reproduced in experimental animal models (3, 5). However, the prevalent hypothesis described above does not provide a complete description of the series of phenomena in the different cells due to the multiple cell types and organs involved in sepsis and septic shock.

The paper "Hypoxia accelerates nitric oxide-dependent dysfunction of mitochondrial complex I in activated macrophages" by Frost et al. (12) in this issue of the *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology* focuses on activated macrophages and describes a decreased complex I activity and whole cell respiration and the enhancing effect of reduced O<sub>2</sub> concentrations. The paper reports that LPS/IFN- $\gamma$ -activated macrophages show a very marked increase ( $\sim$ 30 times) of the primary NO production and a marked increase ( $\sim$ 7 times) of the NO metabolites, peroxynitrite and nitrite.

Macrophage respiration was significantly decreased after cell activation with LPS/IFN- $\gamma$ , and the effect was partially prevented by GSH and markedly inhibited by a nitric oxide synthase (NOS) competitive inhibitor (L-N<sup>G</sup>-1-iminoethyl-ornithine; L-NIO). The inhibitory effect of LPS/IFN- $\gamma$  activation on respiration and on complex I activity increased over time and was accelerated by a low-O<sub>2</sub> environment, despite less NO and peroxynitrite being generated. The study by Frost et al. (12) shows an elegant linear correlation between macrophage O<sub>2</sub> uptake and complex I activity at four time points and two O<sub>2</sub> conditions in the 0- to 24-h period. The direct relationship found between cellular O<sub>2</sub> uptake and complex I activity emphasizes the importance of NADH-ubiquinone reductase as a rate-limiting component of mitochondrial respiration in pathological states. Indeed, complex I-decreased activities have been recognized in hereditary and acquired mitochondrial diseases (such as Parkinson's disease) (7) and in normal

senescence (14, 15). The decreased NADH-dehydrogenase activity present in dysfunctional mitochondria generates more reactive oxygen species, primarily superoxide radical by autoxidation of the FMN semiquinone. It was suggested that the oxidative damage produced by free radical reactions is a cause of mitochondrial dysfunction in aging (14, 15).

Frost et al. (12) clearly identified inducible NOS (iNOS) as the upregulated NOS isoform by Western blot. Recently, Alvarez and Boveris (1) reported that diaphragm and heart of LPS-treated rats show a selectively (1.3 to 2.2 times) increased mitochondrial NOS (mtNOS) activity with a lower effect (1.5 to 1.1 times) in the cytosolic NOS. Considering the uncertainty in the specificity of mtNOS antibody reactivity (13), it is possible that macrophage mitochondria increase their mtNOS activity and that the vicinity of NO source and target favor complex I inactivation.

The study by Frost et al. (12) reports an increase in whole cell tyrosine nitration that was inhibited by the NOS inhibitor L-NIO, which was higher when cells were incubated at 21 % O<sub>2</sub> compared with 1% O<sub>2</sub> and that seems to contribute to the mechanism of complex I and whole cell respiration inhibitions. Nitration was initially higher in mitochondria than in the cytosol, although by 24 h, nitration level was higher in the cytosol. The early mitochondrial nitration agrees with the reported nitration of submitochondrial fractions by the activity of mtNOS (11).

NO has three target sites at the mitochondrial respiratory chain in which NO, directly or indirectly after peroxynitrite formation, inhibits electron transfer. The three sites are NADH-dehydrogenase (complex I), ubiquinone-cytochrome *c* reductase (complex III), and cytochrome oxidase (complex IV). Cytochrome oxidase (6, 9) and ubiquinol-cytochrome *c* reductase (17) are directly inhibited by NO. Peroxynitrite, the product of NO and superoxide radical, inhibits in a close-to-irreversible manner both complex III (8) and complex I (10, 18) activities. The study by Frost et al. (12) describes in detail and correlates complex I inhibition with respiratory inhibition in activated macrophages.

Interestingly, NO is the first molecule that fulfills the requirement for a physiological modulator of cytochrome oxidase activity with an O<sub>2</sub>-competitive mode of binding and inhibition. NO is intramitochondrially produced by mtNOS at a significant rate near the target site, and it has been calculated that endogenous mtNOS activity inhibits mitochondrial respiration in the tissues by 18–25% (2). Cytochrome oxidase inhibition should be even more important in tissues or conditions with low O<sub>2</sub> level, such as in inflammation areas. It is then clear that hypoxia enhances both peroxynitrite-dependent complex I inactivation and the reversible and NO/O<sub>2</sub>-competitive inhibition of complex IV. The reversible cytochrome oxidase inhibition by NO was not observed in the conditions in which macrophage respiration was determined in the study by

Address for reprint requests and other correspondence: A. Navarro, Dept. of Biochemistry and Molecular Biology, Faculty of Medicine, Univ. of Cádiz, P. Fragela 9, 11003 Cádiz, Spain (E-mail: ana.navarro@uca.es).

Frost et al. (12); the respiration assay included a cell dilution in the reaction medium, with the corresponding increase in O<sub>2</sub> concentration and decrease in the NO/O<sub>2</sub> ratio, before the measurement with the O<sub>2</sub>-sensitive electrode. The conditions used were particularly convenient to detect the decrease in complex I activity, the main point of entry of reducing equivalents to the mitochondrial respiratory chain, and its relationship to whole macrophage respiration.

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