O13. Real-time analysis of reactive oxygen and nitrogen species released by single immunostimulated macrophages

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As we and others have previously demonstrated, electrochemical techniques offer the possibility of direct, real-time measurements of important biological species released by single cells [1]. We have successfully detected the release of ROS/RNS by single macrophages stimulated by physical membrane depolarization [2] or calcium ionophore [3], using modified carbon microelectrodes (10 μm tip diameter).

In the present study [4], RAW 264.7 macrophages were stimulated with interferon-γ (IFN-γ) and lipopolysaccharide (LPS) in order to induce expression of the inducible isoform of nitric oxide synthase (iNOS, NOS2). The ensuing ROS/RNS release by a single macrophage was then followed in real-time by amperometry. In comparison with untreated macrophages, significant increases in responses were observed for activated macrophages. Nitric oxide ($NO^{\cdot}$), nitrite (NO_2^-) and peroxynitrite ($ONOO^-$) were the main reactive species detected. The amounts of these reactive species were quantified (femtomoles range), and their average fluxes (thousands of molecules/ms range) released by a single, activated macrophage were evaluated.

The detection of $ONOO^-$ is of particular interest, since its role and implications in various physiological conditions have been widely debated. Herein, direct evidence for the formation of $ONOO^-$ in stimulated macrophages is presented. The presence of 1400 W, a selective inducible nitric oxide synthase (iNOS) inhibitor, led to an almost complete attenuation of response from activated RAW 264.7 cells. The majority of the reactive species released by a macrophage are thus likely to be derived from $NO^{\cdot}$ and superoxide ($O_2^{\cdot -}$) co-produced by iNOS.

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O14. On EPR detection of nitric oxide in vivo

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Nitric oxide ($NO^{\cdot}$) is a peculiar radical: Ground state is not paramagnetic ($g = 0$ since orbital and spin magnetic moments cancel); low reactivity with other molecules except superoxide ($O_2^{\cdot -}$); thermodynamically unstable; dimerizes to N_2O_2; difficult to detect in-vivo.

Spin trapping with iron–dithiocarbamate complexes is unique method to detect $NO^{\cdot}$ in tissues [1]: Trapping proceeds in-vivo, but EPR detection of the paramagnetic $NO–Fe^{2+}$-DETC adducts (MNIC) is ex-vivo in frozen tissue biopts.

We will discuss the technique for in-vivo detection of endogenous NO radicals. Recent advances in sensitivity now allow detection of basal (i.e. unstimulated) NO levels even in small tissue sections of young lab animals. We will illustrate the method with three practical examples:

1. Functional improvement of kidney transplants (with UMC Utrecht).
2. Perinatal therapy of spontaneous hypertension (with UMC Utrecht).
3. Nitrite therapy for large area skin burns (with UK Aachen).

Reference

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NO and Mitochondria

O15. Nitric oxide, mitochondria and their dynamics in myogenesis

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Mitochondria exist in two interconverting forms, i.e. as small isolated particles, and as extended filaments, networks or clusters connected with intermitochondrial junctions. Here we provide evidence that in differentiating myoblasts endogenous NO generation controls mitochondrial shape: in the absence of NO mitochondrial fission occurs rapidly. Mitochondrial fusion was instead not modified by NO.

A key protein involved in mitochondrial fission is the large GTPase DRP-1. DRP-1 translocation to the mitochondria and interaction with the specific docking protein hFis-1 promotes mitochondrial fission. DRP-1 translocation and mitochondrial fission were stimulated by L-NAME and inhibited by exogenous NO. The effects of NO depended on generation of cyclic GMP. We also found that in differentiating myoblasts NO is required for the expression of specific myogenic differentiation markers and that its action was mediated via its control on mitochondrial fission. Our results indicate that NO controls a key event in mitochondrial dynamics that may have relevant implications for both myogenesis and control of energy metabolism in developing skeletal muscle.

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O16. Nitric oxide, mitochondria and the cellular response to hypoxia

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The chemical reduction of molecular oxygen during mitochondrial oxidative phosphorylation is the primary source of metabolic energy for all eukaryotic cells. Thus any disruption to oxygen supply represents a serious metabolic threat to continued cell, tissue and whole animal survival. Consequently, over the course of evolution, we have developed the ability to respond to a drop in cellular oxygen levels (hypoxia) with the induction of an adaptive phenotype which is regulated by the hypoxia inducible transcription factor, HIF. Nitric oxide, which inhibits the mitochondrial consumption of oxygen by inhibition of cytochrome *c* oxidase (the terminal enzyme of the electron transport chain) inhibits the activation of HIF in hypoxia through the redistribution of oxygen to cytoplasmic oxygen sensing hydroxylases. Here, we will review the role of NO in the cellular response to hypoxia and discuss implications for diseases ranging from cancer to chronic inflammation.

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O17. NO, mitochondria and cell death

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Nitric oxide (NO) has at least 3 actions on mitochondria that may promote or inhibit cell death:

1. NO inhibits cytochrome oxidase in competition with oxygen, causing a dramatic increase in the apparent K_M of respiration for oxygen, and potentially sensitising cells to hypoxia. We find that NO from nNOS sensitises neurons in culture to hypoxia-induced death, via NO inhibition of neuronal cytochrome oxidase, raising the oxygen requirement for cellular respiration.
2. NO or *S*-nitrosothiols can inactivate mitochondrial complex I, and this can be reversed by light or DTT, suggesting *S*-nitrosylation of the complex. We find that this inactivation causes a large increase in oxidant production by mitochondria, which is reversed by light or DTT.