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# NOVEL METHODS OF DETECTING OXYGEN AND NITROGEN REACTIVE SPECIES

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**Nitric oxide (NO) has been implicated recently in a number of diverse physiological processes, including smooth muscle relaxation, inhibition of platelet adhesion and neurotransmission. Besides these beneficial functions, NO exhibits cytotoxic affect such as inactivating biologically important mitochondrial respiratory enzymes and neutrophil NADPH oxidase. To understand the mechanism by which NO is synthesized by nitric oxide synthase (NOS) in tissues and how NO mediates various physiological responses, it is useful to be able to observe real time NO generation at the site of production. We have shown direct, real-time, in-vivo measurement of nitric oxide (NO) in mice using the water soluble metal chelator complex, N-methyl-D-glucamine dithiocarbamate (MGD), and Fe(II) and the lipophilic reagent, N, N-diethyl-dithiocarbamate (DETC), as monitored by electron paramagnetic resonance (EPR) at L-band. The EPR spectrum from the product [(MGD)2-Fe (II)-NO] or [(DETC)-Fe(II)-NO] was observed noninvasively in lipopolysaccharide (LPS)-treated mice. In-vivo EPR measurements of [(MGD)2-Fe(II)-NO] at several regions in the body (from the head to the tail) indicated that the NO was generated mostly in the upper abdomen near the liver. This was confirmed by ex-vivo EPR measurements on isolated organs where the higher NO levels were detected in the liver and kidney. In the case of [(DETC)-Fe(II)-NO], we were successful in observing relatively high concentrations of NO trapped in excised brain tissue. The spectroscopic results also showed that both the DETC and Fe(II) independently cross the blood brain barrier and combine with NO in the lipid regions of certain parts of the brain. Lastly, the NO-adduct detected in LPS-treated mice brains was not inducible NOS, but probably rather constitutive NOS, since it was not suppressed by the administration, prior to LPS injection, of phenyl N-tert-butyl nitrone (PBN), an inhibitor of the expression of induced nitric oxide synthase (iNOS).**

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