The Identification of Nitric Oxide as Endothelium-Derived Relaxing Factor

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Endothelium-Derived Relaxing Factor From Pulmonary Artery and Vein Possesses Pharmacological and Chemical Properties Identical to Those of Nitric Oxide

Ignarro et al

The identification of endothelium-derived relaxing factor as nitric oxide (NO) dramatically altered the course of vascular biology, as well as other biomedical disciplines. The ubiquity of this natural product of cell metabolism and the complexity of its biochemistry provide a rich source of molecular mediators of phenotype in health and disease.

When Furchgott and Zawadzki first demonstrated in 1980 that the endothelium released a substance that relaxed blood vessels in response to muscarinic agonists, the field of vascular biology underwent a seismic change. The chemical nature of this effector, denoted at that time as endothelium-derived relaxing factor (EDRF), was unknown. Over the next several years, certain properties of EDRF were reported, which moved the field closer to understanding its likely structure. Patterns of vasorelaxation were similar to those of direct nitrovasodilators, except that these agents exerted their effects independent of the endothelium. In 1977, Murad et al had shown that nitrovasodilators and nitric oxide (NO) activate guanylyl cyclase to effect smooth muscle relaxation and vasodilation. Vasorelaxation by EDRF, like that by direct nitrovasodilators, also was shown to be temporally preceded by activation of guanylyl cyclase and cGMP accumulation. Vasorelaxation by EDRF, like that by direct nitrovasodilators, was inhibited by hemoglobin and myoglobin.

In addition, and importantly, Ignarro et al in their classic article used the shift in the Soret peak of the absorption spectrum of deoxyhemoglobin from 433 to 406 nm as evidence for the formation of nitrosyl hemoglobin. This key observation definitively demonstrated that EDRF is NO, a conclusion that was independently reached by Moncada et al by its unique chemiluminescent product with ozone. Because of this essential observation, the senior author of the classic article, Louis Ignarro, received the Nobel Prize in Physiology or Medicine in 1998, together with Robert Furchgott and Ferid Murad.

The discovery that EDRF is NO ushered in a new era of investigation in biomedicine. The ubiquity and dazzling pluripotentiality of this heterodiatomic molecule were rapidly demonstrated as literature reports of its many actions grew exponentially over time. NO is synthesized by the family of NO synthases from L-arginine, yielding citrulline as a coproduct. NO is a free radical, but one that is not as reactive as other biological free radicals, diffusing on average micron distances before engaging in a reaction with another molecule. The structural simplicity of this compound understates the astonishing complexity of reactions that result from its unique biochemistry. One example of this biochemical complexity includes the range of redox states of nitrogen oxides and their physiological relationship to NO itself. Specifically, nitrite and nitrate are higher oxidation states of NO that were previously believed to be stable oxidation end products of the redox sequence. Recent work, however, suggests that these oxidized species can be recycled in vivo in a process that involves oral microbiota and foodstuffs. Oral bacteria can reduce dietary nitrate to nitrite, which, on entering the acidic milieu of the stomach, undergoes nonenzymatic reductive conversion to NO. In parallel, nitrate and nitrite in blood that originate from the diet and from systemic production and oxidation of NO can be taken-up actively by the salivary glands, whereby it enters the nitrate reductase–rich bacterial milieu of the oral cavity, facilitating the cycle (Figure 1).

A second example of this biochemical complexity is the multiple pathways within the complex metabolism of NO by which S-nitrosothiols form, including S-nitrosothiols. S-nitrosothiols comprise an ever-increasing pool of species...
that stabilize NO, minimizing its oxidative inactivation and promoting its many biological actions. Multiple lines of evidence also suggest that S-nitrosothiols may, in many instances, be the direct proximate mediator of EDRF-like effects.\textsuperscript{11–13} In addition, and importantly, S-nitrosylation of the proteome is a unique form of posttranslational modification that can have significant consequences for protein function and cell phenotype. S-nitrosothiols can form by the reaction of the thiol moiety with dinitrogen trioxide (N\(_2\)O\(_3\)), peroxynitrite anion (ONOO\(^-\)), or nitrosonium ion (NO\(^+\)), or by trans-S-nitrosylation, as illustrated in Figure 2.\textsuperscript{14} Furthermore, S-nitrosothiols also can form in the acidic environment of the stomach from nitrite (acidified nitrite) and thiols or mediated via hemoglobin in blood. This and other biochemical pathways parallel the wide-ranging cellular and physiological effects of NO, leading to its designation as the molecule of the year by Science in 1992.

Given the structural simplicity of NO and its ubiquity, it is reasonable to ask why it took so long to recognize that it is a normal product of eukaryotic cell metabolism. One response to this question is that the biochemistry is highly versatile, complicating detection of the biologically active species. In addition, the steady-state concentrations of NO are low in vivo compared with those of its higher oxidation states, and the available assays have been insensitive, limiting its detection. However, this seems an unlikely explanation because reasonable analytical methods for detecting NO have been available for decades. Hermann\textsuperscript{15} first demonstrated the interaction of NO with (a component of) blood in 1865, which was highlighted by the formation of a bright red color. This color change was not unlike that caused by the reaction of carbon monoxide with blood (later recognized as the formation of carboxyhemoglobin) and probably led to the erroneous diagnosis of carbon monoxide poisoning in cases of overwhelming sepsis caused by nitrifying organisms reported as early as 1925.\textsuperscript{16} Spectrophotometric detection of nitrosyl hemoglobin was first reported in 1925 by Anson and Mirsky,\textsuperscript{17} and this technique was subsequently used to demonstrate the presence of NO in the urine of a patient with hemuria and bacteriuria in 1955.\textsuperscript{18} With the advent of electron paramagnetic resonance spectroscopy and its application to biological systems, nitrosyl hemoglobin was demonstrated in the blood of mice and rabbits exposed to inhaled NO by monitoring the appearance of the hyperfine triplet at \(g \approx 2.0\).\textsuperscript{19} This study focused on the interaction of NO with hemoglobin as a potential marker of the in vivo toxicity of the inhaled gas. Interestingly, in one figure in that article, the kinetics of the formation and decay of nitrosyl hemoglobin is plotted after exposure to 10.6 ppm of NO. In the steady-state, \(\approx 0.13\%\) of total hemoglobin is converted to nitrosyl hemoglobin. With discontinuation of the inhaled NO, the nitrosyl hemoglobin decayed but persisted at detectable levels of \(\approx 0.01\%\) to 30 minutes or 81.8 half-lives,\textsuperscript{20} well beyond what one would predict if there was not some other endogenous source of NO to maintain the new steady-state. The authors commented on this unexpected finding,
stating that “…even after 30 minutes [nitrosylhemoglobin] was still present.” Thus, these and other data hinted at the existence of NO or an NO-like species as a product of normal mammalian metabolism. Yet, it would take another 12 years before the endogenous production of NO was definitively proven in this classic article in the Journal, ushering in a very broad field in biomedicine that continues to be a rich source of discovery.

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References
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