

This Week's Citation Classic®

Palmer R M J, Ferrige A G & Moncada S. Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. *Nature* 327:524-6. 1987. [Wellcome Research Laboratories, Beckenham, Kent, England]

This paper showed, using a combination of biological assay and chemical measurement, that vascular endothelial cells release nitric oxide in amounts sufficient to account for the vascular relaxation caused by endothelium-derived relaxing factor. As such, it was the first demonstration that mammalian cells synthesize nitric oxide to act as a biological mediator. [The SC[®] indicates that this paper has been cited in more than 1.650 publications.]

Cellular Nitric Oxide Synthesis

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That nitric oxide (NO), a gas previously recognized as an atmospheric pollutant, is synthesized by mammalian cells for specific biological functions was a totally unexpected discovery that still remains surprising. Furthermore, we still do not know whether NO is the only such gaseous agent or is one of a number of gases yet to be identified as playing biological mediator roles. These are some of the main reasons why our 1987 paper demonstrating the synthesis of NO from vascular endothelium has generated such wide interest and has now become a *Citation Classic*. Furthermore, this finding has opened up a whole new area of biological research.

First, we demonstrated the biological similarity between NO gas and the so-called endothelium-derived relaxing factor (EDRF), a substance previously discovered by R.F. Furchgott and J.V. Zawadzki in 1980.¹ Conclusive evidence that EDRF was indeed NO required the demonstration of the release of NO by endothelial cells. A chemiluminescence method for measuring NO had previously been developed to determine nitrite levels in foods and nitrogen oxides in exhaust emissions and atmospheric pollutants.

We found that the Food Science Laboratory of the University of Surrey had such a chemiluminescence machine and talked to C.L. Walters, the head of the unit, who allowed us to use it.

In this way, we rapidly gathered information clearly showing that bradykinin was able to release very small quantities of NO from vascular endothelial cells in culture and, in a long day of work just before the Christmas of 1986, we succeeded in generating a recording that was definitely convincing. In the meantime, Tony Ferrige modified the chemiluminescence method in order to increase its sensitivity, enabling us to produce the recordings that were subsequently published.

We went on the following year to show that the NO was synthesized from L-arginine² and that N^G-monomethyl-L-arginine was a specific inhibitor of NO synthesis. This compound, used as a pharmacological and biochemical tool, has contributed greatly to the understanding of the many different biological roles of NO. Our further discovery³ that the L-arginine:NO pathway also existed in the central nervous system led us to suggest that this was a widespread mechanism for the regulation of cell function and communication.^{5,6}

NO is now recognized as an important regulator of blood pressure, platelet activation, and peripheral and central neurotransmission. These actions are all brought about by NO acting as the endogenous activator of the soluble guanylate cyclase, resulting in elevation of cyclic GMP levels. Nitric oxide is also a cytotoxic effector molecule in host defense against pathogens and tumor cells. This occurs via inactivation by NO of iron-sulfur-centered enzymes involved in respiration and cell division in target cells. Furthermore, when released in large quantities, NO contributes to pathophysiological conditions such as the hypotension of endotoxin shock. The measurement of products of NO synthesis, using some of the methodology originally developed in the 1987 *Nature* paper, is also yielding very exciting information about human physiology and pathophysiology and is pointing towards novel therapeutic opportunities.

1. Furchgott R F & Zawadzki J V. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature* 288:373-6. 1980. (Cited 2,545 times.)
2. Palmer R M J, Ashton D S & Moncada S. Vascular endothelial cells synthesize nitric oxide from L-arginine. *Nature* 333:664-6. 1988. (Cited 750 times.)
3. Palmer R M J, Rees D D, Ashton D S & Moncada S. L-arginine is the physiological precursor for the formation of nitric oxide in endothelium-dependent relaxation. *Butchem. Biophys. Res. Commun.* 153:1251-6. 1988. (Cited 380 times.)
4. Knowles R G, Palacios M, Palmer R M J & Moncada S. Formation of nitric oxide from L-arginine in the central nervous system: a transduction mechanism for stimulation of soluble guanylate cyclase. *Proc. Nat. Acad. Sci. USA* 86:5159-62, 1989.
5. Moncada S, Palmer R M J & Higgs E A. Biosynthesis of nitric oxide from L-arginine: a pathway for the regulation of cell function and communication. *Biochem. Pharmacol.* 38:1709-15. 1989.
6. ----- . Nitric oxide: physiology, pathophysiology and pharmacology. *Plumtncv Rev.* 43:109-42. 1991.

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