Nitric oxide: inducer or suppressor of apoptosis?

Pierluigi Nicotera, Bernhard Brune and Giacinto Bagetta

Among the molecules involved in cell signalling nitric oxide (NO) in its different chemical forms has perhaps the most diverse action. Nitric oxide is both a rapid messenger and a cytotoxic mediator. Nitric oxide-mediated reactions seem to utilize a relatively common biochemical trigger, i.e. the *S*-nitrosylation or nitration of proteins; this mechanism is responsible for the regulation of an impressive number of normal and abnormal functions in biological systems. On the one hand, as a neurotransmitter, NO regulates intestinal peristalsis, autonomic and neuroendocrine functions and plays a role in the regulation of behaviour[1]. On the other hand, overwhelming production of NO, downstream to prolonged stimulation of glutamate receptors, has been implicated in excitotoxic neuronal injury[2]. The latter may be causally involved in the pathogenesis of neurological disorders such as brain ischaemia, Alzheimer's and Parkinson's diseases. The effects of NO on vascular tissue were recognized at a very early stage[3] and the role of NO and its congeners as vasodilators is well established[1]. Meanwhile, it has become clear that NO may play important roles in immune cell regulation, in spermatogenesis, in the pathogenesis of some viral infections, in liver injury and as a plant growth regulator[4]. Neuronal differentiation and cell growth in general are also affected by NO, which can also elicit or prevent apoptosis in a variety of *in vitro* systems[5].

It is the role and mechanisms of NO-induced cell death, apoptosis or necrosis that has attracted growing interest over the past few years. This interest was reflected in a conference on *Nitric Oxide and The Cell**, where the roles of NO in cell death were widely discussed. Overviews on the general signalling mechanisms that underlie the central role of NO in physiology and pathophysiological reactions (Salvador Moncada, University College London) and the recent understanding that *S*-nitrosylation reactions can mediate both physiological activation and adaptive stress responses (Jonathan Stamler, Duke University Medical Center, North Carolina) have introduced the concept that NO in its various forms can elicit or suppress the natural cell death programme, apoptosis. It has become clear that, depending on its concentration, the biological redox milieu and the involvement or induction of intracellular defence mechanisms, NO can either suppress apoptosis and eventually stimulate proliferation or activate the cell death programme.

Nitric oxide seems to play an active role in the development of the nervous system (Natalia Peunova, Cold Spring Harbor Laboratory, New York) and of the eye in *Drosophila* (Grigori Enikolopov, Cold Spring Harbor Laboratory, New York). It also promotes differentiation of immature neurones eliciting growth arrest and is a modulator of nerve growth factor (NGF) survival function. In *Drosophila*, NO acts as an antiproliferative agent during cell differentiation and inhibition of nitric oxide synthase (NOS) results in surplus cell proliferation with excessive growth.

Overwhelming generation of NO can also result in the activation or suppression of apoptosis in postmitotic adult neurones and in various cell lines[6,7]. Neuronal loss in several neurodegenerative disorders may also occur by excess apoptosis. Slow excitotoxic mechanisms or alteration of growth factor responses may lead to a progressive loss of neurones and to cognitive or motor dysfunction. A novel mechanism to explain apoptosis of cerebellar granule neurones elicited by NO donors has been described by Pierluigi Nicotera (University of Konstanz, Germany). Nitric oxide donors or ONOO elicit apoptosis in cerebellar granule cells by promoting exocytosis of an *N*-methyl-D-aspartate (NMDA) receptor agonist which would act intrasynaptically on this receptor. In this system, NO-induced apoptosis is entirely blocked by agents that directly or competitively block the NMDA receptor channel or by others that interfere with exocytosis. The possibility was discussed that *S*-nitrosylation of proteins involved in vesicular transport of neurotransmitters such as SNAP-25 (synaptic-associated protein-25) may lead to enhanced release of excitatory amino acids that, in turn, trigger autocrine excitotoxic death. This mechanism may be relevant to perpetuating slow excitotoxicity in damaged brain areas.

Another relevant aspect of brain pathology that has attracted increasing interest over the past few years is the onset of dementia in AIDS patients. While neurones do not seem to be infected by the HIV virus, viral components such as gp120 coat protein may cause brain damage by eliciting excitotoxic reactions[8]. These include stimulation of glutamate receptors and increased NO production via Ca^{2+}-dependent activation of brain NOS (bNOS)[8]. However, treatment of rats with intracerebroventricular infusion of recombinant gp120 reduces bNOS expression (Giacinto Bagetta, University of Calabria, Italy) in brain areas (i.e. hippocampus) involved in memory formation with no apparent effect on bNOS expression in the cerebral cortex where the treatment produces apoptosis. This would make unlikely a direct involvement of bNOS in neuronal apoptosis after gp120 treatment. Notably, other brain areas, such as the hippocampus, seem to be resistant to gp120-induced apoptosis. This may be the result of increased NGF production, which would prevent the onset of apoptosis. The differences between *in vivo* and *in vitro* models for the

P. Nicotera, Professor of Molecular Toxicology, Faculty of Biology, University of Konstanz, Box 5560-X911, D-78434 Konstanz, Germany.
B. Brune, Associate Professor of Experimental Medicine, Faculty of Medicine, Department IV – Experimental Division, University of Erlangen-Nurnberg, Loschgest 8, D-91054 Erlangen, Germany, and
G. Bagetta, Professor of Pharmacology, Head of Department, Department of Pharmaco-Biology, University of Calabria, Arcavacata di Rende, Cosenza, Italy.

**2nd International Paraelios Symposium on Nitric Oxide and The Cell, 15–17 September 1996, Calabria, Italy.*

 0165 – 6147/97/$17.00 PII: S0165-6147(97)01070-5

study of gp120 neurotoxicity were emphasized by Gerry Melino (University of L'Aquila, Italy), who showed that NMDA and gp120 can elicit cell death *in vitro* in neuroblastoma cells. The role of inhibitors of Ca^{2+}-dependent cystein proteases in preventing neuronal loss in this system was also described.

In other cell systems, NO can also induce or suppress apoptosis. NO induces macrophage apoptosis, which is associated with upregulation of the tumour suppressor gene, p53 (Bernhard Brune, University of Erlangen-Nurnberg, Germany). In this system, induction of iNOS (inducible NOS) leading to NO accumulation causes the typical morphological and biochemical alterations of apoptosis including the activation of proteolytic system collectively known as caspases that cleave nuclear proteins such as poly ADP (ribose) polymerase (PARP). Overexpression of the anti-apoptotic gene, Bcl-2 (B-cell leukaemia oncogene-2) suppresses PARP cleavage without affecting the accumulation of p53. This suggests that the controlling function of Bcl-2 is downstream to the induction of p53 and regulates the intervention of executors of apoptosis such as caspases. Altered expression of surface molecules or their links with cytoskeletal elements is also a feature of apoptotic cells. A possible role of integrins as regulators of apoptosis has been described by Eduardo Lapetina (Burroughs Wellcome, North Carolina), who has shown data on the role of mitogen-associated protein and JNK (*c*-jun NH_2-terminal kinase) kinases as potential modulators of NO-induced apoptosis. Finally, induction of apoptosis in pancreatic β-cells may underlie the initial loss of cells in type I diabetes. Formation of cGMP and activation of protein kinase C may be essential components in this response (Anne Loweth, Keele University, UK).

Under certain circumstances, NO can inhibit apoptosis. Upregulation of iNOS for example, prevents apoptosis elicited in an *in vivo* model of tumour necrosis factor- plus galactosamine-induced liver injury (Timothy Billiar, University of Pittsburgh, Pennsylvania). The contrasting effects of NO may depend on its concentration and in the case of the protective effect, it would involve either the induction of the heat shock 70 response or the activation of the soluble guanylate cyclase. Another condition whereby NO can prevent apoptosis seems to involve upregulation of intracellular antioxidant systems, especially glutathione (J. Ponte, University of Vermont, USA). These data again emphasize the relevance of the intracellular redox state for the selection of positive or adverse effects of NO.

Concluding remarks

In conclusion, the dual role of NO as mediator or suppressor of cell death has been highlighted by many presentations in this meeting. The possibility that the expression of NOS in different cell systems and that enhanced NO generation can contribute to the protection or vulnerability of cells to toxicity is an open question to be addressed by future development in this area.

References

1 Moncada, S., Palmer, R. M. J. and Higgs, E. A. (1991) *Pharmacol. Rev.* 43, 109–142
2 Dawson, V. L., Dawson, T. M., London, E. D., Bredt, D. S. and Snyder, S. H. (1991) *Proc. Natl. Acad. Sci. U. S. A.* 88, 6368–6371
3 Palmer, R. M. J., Ferrige, A. G. and Moncada, S. (1987) *Nature* 327, 524–526
4 Moncada, S., Stamler, J., Gross, S. and Higgs, E. A. (1995) *The Biology of Nitric Oxide* (Pt 5), Portland Press
5 Nicotera, P., Bonfoco, E. and Brune, B. (1995) *Adv. Neuroimmunol.* 5, 411–420
6 Bonfoco, E., Kraine, D., Ankarcrona, M., Nicotera, P. and Lipton, S. A. (1995) *Proc. Natl. Acad. Sci. U. S. A.* 92, 7162–7166
7 Dimmeler, S., Haendeler, J., Nehls, M. and Zeiher, A. M. (1997) *J. Exp. Med.* 185, 601–607
8 Lipton, S. E. and Gendelman, H. E. (1995) *New Engl. J. Med.* 332, 934–940

From the Societies…

EUPHAR

Federation of European Pharmacological Societies, International Symposium :

DRUG TARGETS IN HEART AND BRAIN ISCHEMIA
Florence (Italy) July 11-14, 1997

This symposium aims to promote innovative dialogue among pharmacologists, cardiologists and neurologists, both at basic and clinical levels of research. Knowledge of the degenerative and cell death mechanisms in the nervous system may provide important clues to intervention within the cardiovascular system. In turn, the extensive experience of clinical and emergency management of cardiac ischemia may provide models and tools for prevention and treatment of brain ischemia.

Chairpersons:
S. Moncada (UK), B.K. Siesjo (Sweden), R. Paoletti (Italy)

Sponsored by:
Fedaration of European Pharmacological Societies (EUPHAR)
International Stroke Society
Foundazione Giovanni Lorenzini Medical Science Foundation
Giovanni Lorenzini Medical Science Foundation
Institute of Pharmacological Sciences, University of Milan