Mini Review

Nitric Oxide as a Bifunctional Regulator of Apoptosis

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It was inevitable that important relationships between two of the most intensely studied topics in biomedical research, apoptosis and nitric oxide (NO), would become apparent. Apoptosis is essential to normal development as well as physiological cell turnover. Although apoptosis in excess can manifest as tissue damage, a failure to undergo apoptosis constitutes pathological cellular overgrowth. It is now evident that NO and its reaction products can either promote or prevent apoptosis in a multitude of settings. The ubiquitous distribution of the NO synthases and the remarkable diffusibility and diverse chemical reactivity of NO in biological systems make this molecule unique among the regulators of apoptosis. Understanding the factors that govern the consequences of NO exposure on cell viability and identifying the conditions in which NO regulation of apoptosis contribute to pathology are topics of considerable interest and potential importance. In this article, we will review the recent observations on NO as a regulator of apoptosis.

Apoptosis, or programmed cell death, is distinguished from lytic or necrotic cell death by specific biochemical and structural events (see recent review in Reference 1). Apoptotic signals trigger cell-specific signaling pathways, including protease activation, followed by the appearance of morphological changes characteristic of cells undergoing apoptosis, including condensation of nuclei and cytoplasm, blebbing of the cytoplasmic membranes, and finally fragmentation into apoptotic bodies that are phagocytosed by neighboring cells. The elicitation of the signaling events in apoptosis is occurring at a rapid pace and includes the identification of the key roles of cysteine proteases (known as caspases), Bcl-2 family members, and mitochondria.

Caspases, the mammalian counterpart of ced-3 in Caenorhabditis elegans, are a family of cysteine proteases now known to contain at least 14 homologs. Ectopic expression of any of the caspase family proteases can cause apoptosis; however, not all caspase family proteases have been definitively linked to apoptosis. Caspase family genes encode proenzyme forms that require proteolytic cleavage for activation. Caspases can propagate apoptotic signaling by cleaving/activating other caspases, or they can execute the terminal events in apoptosis by cleaving key death substrates. For example, caspase-9 cleaves/activates caspase-3, whereas caspase-3 cleaves specific target proteins, including poly(ADP-ribose) polymerase (PARP), DNA-dependent kinase, and the inhibitor of the caspase-dependent activated deoxyribonuclease (ICAD). The antiapoptotic effect of compounds that inhibit either the activation or activity of caspase-3–like proteases suggests that apoptosis can be regulated by modification of the protease-signaling cascade. One mechanism by which Bcl-2, itself a substrate for caspase-3, prevents cell death during physiological and pathophysiological processes is through the inhibition of mitochondrial cytochrome C release. The release of cytochrome C results in the activation of caspase-9. Although endogenous inhibitors of caspase activation and activity have been described, none has been shown to be more prevalent than NO.

NO is short-lived and is synthesized from L-arginine by the catalytic reaction of NO synthases (NOSs) (reviewed in Reference 2). NOSs are expressed in microorganisms, plants, and mammals, in addition to participating in diverse physiological functions including neurotransmission, regulation of vascular tone, cellular communication, inflammation, and immune responses. The mammalian NOS isoforms include the neuronal type 1 isoform (nNOS), the inducible form type 2 (iNOS), and the endothelial type 3 (eNOS). nNOS and eNOS are constitutively expressed enzymes and are regulated predominantly at the posttranslational level. iNOS is constitutively expressed in only a few cell types but can be induced in essentially every cell when appropriately stimulated. Rate of transcription is one important level of regulation for iNOS. The comparatively small amount of NO produced by constitutive NOS in endothelial and neuronal cells is important for cellular signaling events such as blood pressure regulation and neurotransmission. The much larger amount of NO generated by iNOS functions as both a regulator and effector during infection and inflammation. One effector function includes direct cytotoxicity toward tumor cells, microorganisms, and host cells. The cytotoxic capacity of NO has been confirmed in numerous systems using diverse cell targets. In many circumstances, the cytotoxicity is the result of the interaction of NO with superoxide to form peroxynitrite, a potent oxidant. The cytotoxicity of NO produced by iNOS as well as by nNOS has been the topic of intense study for more than a decade; however, more recently, the potent antiapoptotic activity of NO has also received a great deal of attention.
Proapoptotic Effect of NO
The importance of NO-mediated cytotoxicity has been appreciated since the L-arginine→NO pathway was first identified in macrophages. The capacity of NO to induce apoptosis was first appreciated by Albina et al., who showed that NO caused apoptosis in macrophages. Since then, several cell types have been shown to undergo apoptosis in response to NO or peroxynitrite. Primary cell types that are particularly sensitive include macrophages, pancreatic islets, thymocytes, and certain neurons. The proapoptotic effect on these cells seems to be independent of cGMP accumulation; however, NO has been shown to induce apoptosis via the stimulation of soluble guanylyl cyclase in vascular smooth muscle cells in vitro.

Although the factors that determine cell-specific sensitivity to NO-mediated apoptosis are not clearly elucidated, the induction of apoptosis by NO can be the result of DNA damage. DNA damage results in the accumulation of the tumor suppressor protein p53, which has been described as an essential and early indicator of NO-induced apoptosis. p53, in turn, induces cell cycle arrest by upregulating p21 or apoptosis. The induction of apoptosis often requires exposure to high levels of exogenous NO donors. Short-term exposure to high levels of NO may overwhelm natural protective pathways, leading to the activation of apoptotic signaling pathways. Such toxic levels of NO may have limited relevance to the in vivo situation. Another important factor in the susceptibility to NO is whether a cell type has the capacity to use NO for protection. For example, some cells are protected by cGMP; therefore, cells that possess this signaling pathway may be protected by NO.

NO can interact with superoxide anion to produce the potent oxidant peroxynitrite. Some studies suggest that the proapoptotic effect of NO is a result of the formation of peroxynitrite, inducing apoptotic DNA fragmentation and p53-dependent apoptosis. The formation of peroxynitrite is determined by the ratio of NO to superoxide, and the cellular susceptibility to peroxynitrite is dependent in large part on the levels of antioxidants (eg, thiols).

Antiapoptotic Actions of NO
In view of the extensive literature describing NO as a cytotoxic effector, it is not surprising that NO, especially iNOS-generated NO, was rapidly accepted as a potent inducer of apoptosis. A seminal paper by Mannick et al. in 1994, however, forced a paradigm shift. These authors showed that endogenous iNOS expression or exposure to low doses of NO donors inhibited apoptosis in human B lymphocytes. Following this report, similar findings were described in several in vitro cell culture systems including splenocytes, eosinophils, endothelial cells, hepatocytes, and cell lines. In addition, animal experiments demonstrated that lipopolysaccharide (LPS)-induced hepatic apoptosis was increased by administration of NOS inhibitors, and administration of a liver-specific NO donor almost completely blocked the massive hepatic apoptosis induced by tumor necrosis factor (TNF) plus d-galactosamine administration. Several general aspects of NO-mediated inhibition of apoptosis warrant comment. First, NO has been shown to inhibit apoptosis both in vitro and in vivo in certain cell types.

Second, NO inhibits apoptosis induced by many different stimuli, including growth factor withdrawal, TNF, or Fas. Third, multiple mechanisms for the inhibition of apoptosis by NO may exist in a single cell type. For example, NO blocks apoptosis in hepatocytes both via cGMP-mediated interruption of apoptotic signaling and direct inhibition of caspase activity. Finally, the levels of NO generated by either constitutive or iNOS can inhibit apoptosis. In fact, in endothelial cells, constitutive eNOS was adequate to inhibit TNF-induced apoptosis while overexpression of iNOS also effectively suppressed LPS-induced apoptosis without toxicity.

Antiapoptotic Mechanisms
The reactivity of NO in biological systems is complex and permits NO to exert a wide range of actions. Studies on the antiapoptotic mechanisms of NO have identified a series of NO target interactions that range from indirect and nonspecific to direct interaction with apoptotic machinery.

Induction of Cytoprotective Stress Proteins
NO can oxidize intracellular reduced glutathione and thereby change the antioxidant levels within the cell, resulting in oxidative or nitrosative stress. This action stimulates the induction of heat shock proteins HSP32 (heme oxygenase) and HSP70, which protect cells from apoptotic cell death induced by TNF plus actinomycin D and oxidative or nitrosative stress. The molecular mechanism underlying antiapoptotic effect by NO-mediated HSP expression may be associated with two possibilities. The first is the direct suppression of apoptotic signal transduction involving the inhibition of caspase family protease activation. The second involves the chaperon-mediated import of precursor proteins into mitochondria by HSPs. This action controls mitochondrial function and membrane permeability thereby preventing the release of cytochrome C that is required for further activation of caspases.

cGMP-Dependent Inhibition of Apoptotic Signal Transduction
One of the first molecular targets to be identified for NO was the heme protein soluble guanylyl cyclase. NO activates guanylyl cyclase by interacting with its heme and generates cGMP from GTP. Intracellular elevation of cGMP decreases cellular Ca2+ concentration, which is one of the key signals of apoptosis. In some cell types shown to be protected by NO (including hepatocytes, neuronal PC12 cells, and splenocytes), cGMP prevents apoptotic cell death. The molecular mechanisms underlying the NO/cGMP inhibition of apoptosis could involve the activation of cGMP-dependent protein kinase and the inhibition of caspase activation. The inhibition of caspase activation may then limit Bcl-2 degradation and thus explain the increase in Bcl-2 levels observed in splenocytes exposed to NO or cGMP (Figure). However, the mechanism by which cGMP or G kinase suppresses apoptotic signaling remains unknown.

Suppression of Caspase Activity
All caspase proteases contain a single cysteine at the enzyme catalytic site that is essential for activity. For caspase-3, the
Inhibition of Cytochrome C Release

Recent studies have shown that cytochrome C release from mitochondria is a key component in the activation of caspases. 1 Although it is known that Bcl-2 can inhibit cytochrome C release, we have observed that cytochrome C release can also be inhibited by the NO pathway. 26 Furthermore, Bcl-2 cleavage can be inhibited by the caspase-3-like inhibitor Ac-DEVD-cho and/or NO, suggesting that activated caspase-3-like proteases are responsible for Bcl-2 protein cleavage and the inactivation of the antiapoptotic function of Bcl-2. 26 By interrupting this step, NO appears to suppress cytochrome C release, which is a key factor for the amplification of apoptotic signaling through caspase-9 (Figure).

Conclusion

The decision for a cell to undergo apoptosis is the result of a shift in the balance between the antiapoptotic and proapoptotic forces within a cell. The accumulated data indicate that physiologically relevant levels of NO contribute to this balance by suppressing the apoptotic pathway at multiple levels and by several mechanisms (Figure). Inhibition of caspase activity by S-nitrosylation is the best-characterized mechanism for the inhibition of apoptosis by NO and is likely to be effective in cells that can efficiently carry out S-nitrosylation. Higher rates of NO production overwhelm cellular protective mechanisms and shift the balance toward apoptotic death in some cell types. The presence of superoxide may also divert NO to a toxic pathway by leading to the formation of peroxynitrite. Further studies should continue to elucidate the many factors that determine whether NO promotes or inhibits apoptosis.

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References


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