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BRIEF REVIEW

Nitric Oxide: Cytotoxicity versus Cytoprotection — How, Why, When, and Where?

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Nitric oxide (NO) has been found to play an important role as a signal molecule in many parts of the organism as well as a cytotoxic effector molecule of the nonspecific immune response. It appears paradoxical that NO on one side acts as a physiological intercellular messenger and on the other side may display cytotoxic activity *in vivo*. To make things even more complicated, cytoprotective properties of NO are also described. We here review the current understanding of cytotoxic versus cytoprotective effects of NO in mammalian cells and try to highlight the janus-faced properties of this important small molecule.

1. CHEMISTRY OF NITRIC OXIDE

The signal molecule NO[2] is synthesized on demand, after enzyme activation, by constitutively expressed NO synthases (NOS) for short periods of time (seconds to minutes). The killer molecule NO is synthesized by an inducible NOS that, once expressed, produces NO for long periods of time (hours to days). According to calculations, the major differences between cNOS and iNOS activities do not reside in the concentrations of NO generated per enzyme, but rather in the duration of NO produced (1). In addition, the iNOS protein content in fully activated cells may be higher than the cNOS content. Thus, cytotoxicity usually correlates with the product of iNOS and not with the product of the two cNOS (with possible exceptions in brain injury). Thus, regulated pulses versus constant unregulated NO synthesis differentiates between the messenger and the killer properties of NO.

Although being a radical, NO has quite a long life in biological environments, depending on its own as well as concentrations of oxygen and/or other components of the solvent (for review see Ref. 2). In addition, reaction with glutathione, or proteins containing reduced cysteine moieties, yields *S*-nitrosothiols, which are more or less unstable (half-lives: minutes to hours) and decompose, again yielding NO. These can thus be regarded as NO-storage compounds. It is, therefore, impossible to determine exactly the life span of NO in biological systems.

Concerning the cytotoxic effects of NO, one must ask if the effect is really due to nitrogen monoxide. Concerning the physiological signaling function of NO, we can be certain that we deal with NO as the target molecule. The soluble guanylate cyclase is ac-

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[2] Abbreviations used: NO, nitric oxide; NOS, NO synthase; PARP, poly(ADP-ribose)polymerase; LDL, low-density lipoprotein.

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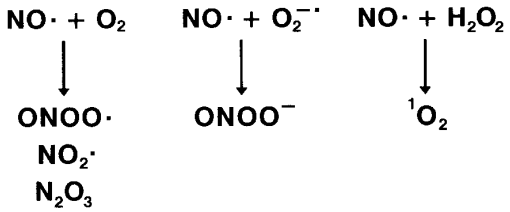

FIG. 1. Reaction products of NO with oxygen or reactive oxygen intermediates.

tivated by NO with an apparent K_M in the nanomolar range and not by other related compounds (3). Concerning the toxic effects of NO, the picture is less clear, especially under the aerobic conditions of cellular life. In addition, activated cells like macrophages, neutrophils, or endothelial cells secrete reactive oxygen intermediates, like H_2O_2, $O_2^{-\bullet}$, $OH^{\bullet}$, and singlet oxygen ($^1O_2$), thereby complicating the local environmental situation. Reaction of NO with H_2O_2 yields $^1O_2$ (4) and a cooperative toxic action of NO and H_2O_2 has been demonstrated (5–7). $O_2^{-\bullet}$ and NO may react to form the peroxynitrite anion ($ONOO^-$), a strong oxidant with a half-life of less than a second. However, macrophage generation kinetics of $O_2^{-\bullet}$ is different from kinetics of NO generation and the amount of $ONOO^-$ generated extracellularly by activated macrophages and endothelial cells *in vitro* appears to be very small (8, 9). In addition, NO seems to inhibit $O_2^{-\bullet}$ production by activated neutrophils by either decreasing NADPH activity or assembly (10–12). The NO oxidation or reduction products, NO^+ and NO^-, respectively, probably do not play a significantly role in biological systems, as NO cannot be oxidized to NO^+ *in vivo* (13). Additionally, both NO^+ and NO^- are highly reactive and would disappear rapidly if formed (13). In contrast, reaction pathways of NO with molecular dioxygen yielding nitrogen dioxide (NO_2), the peroxynitrite radical ($ONOO^{\bullet}$), dinitrogen trioxide (N_2O_3), and/or other compounds are likely to occur *in vivo* (14, 15). In conclusion, NO secreted by activated cells appears to be a complex "cocktail" of substances (Fig. 1). Nevertheless, synthesis of true NO is the necessary first step for creating these compounds.

What concentrations of NO are cytotoxic? It has been calculated that a steady-state concentration of about 4–5 μM NO can be reached in the immediate vicinity of a cell monolayer (1). The diffusion distance of NO secreted by a single cell is surprisingly large (150–300 μm in 4–15 s) and is further potentiated when cells are located in clusters (16, 17). Activated macrophages or islet endothelial cells lyse 100% of syngeneic islet cells within 15 h at a target:effector cell ratio of 1:2 and produce about 15–40 μM NO within that time (18, 19). Using NO donors, concentrations of 0.5–1 mM are necessary to achieve the same cytotoxic outcome (20). However, there is a big difference using cellular or chemical sources of NO. While macrophages or endothelial cells are located and produce NO in the closest neighborhood of target cells, NO donors generate NO throughout the whole culture supernatant. Thus, most of the NO generated is probably autooxidized before it reaches its target. Therefore, it is next to impossible to give an exact answer as to which concentrations of NO are toxic for cells.

NO may react with proteins and nucleic acids. In addition to binding to heme groups, e.g., of guanylate cyclase, hemoglobin, and cytochrome c oxidase, NO theoretically may react with nucleophilic centers like sulfur, nitrogen, oxygen, and aromatic carbons. The prime target for covalent binding of NO to functional groups in proteins under physiological conditions in the presence of oxygen is the SH group (21–24). Tryptophan is the only noncysteine residue that undergoes nitrosation by NO. However, this reaction is about 10-fold slower than S-nitrosylation and thus cannot compete favorably with cysteine in a spontaneous nitrosation reaction (24). Deamination reactions of protein amino groups by NO have been proposed, but not shown (25). N-nitrosation of secondary NH_2 groups, e.g., of lysin moieties, and subsequent deamination reactions could not be observed under physiological conditions, even when high NO concentrations were used (21; K. D. Kröncke, unpublished observations). Nitration of tyrosine residues by NO has been proposed also, but could not be confirmed (22). NO has been shown to N-nitrosylate primary arylamines of nucleotides and subsequent hydrolysis yields deaminated nucleotides (26, 27). However, the selectivity ratio is at least 10^6 times greater for sulfhydryl-containing peptides than for exocyclic amines of DNA bases (21).

TABLE I

Putative Intracellular Transport Forms of Nitric Oxide

	Ref.
Dinitrosyl–iron(II) complexes (DNIC's)	28
N^G-hydroxy-L-arginine–NO adduct	29
Glutathione–NO complex (GSNOH)	30

2. MOLECULAR NO TARGETS IN CELLS

Prior to discussing potential targets of NO within cells, an unsolved mystery must be discussed. While active cNOS is a membrane-bound protein, active iNOS is located in the cytosol. How does the iNOS product NO escape from the cytosol of the producer cell without reacting with the many intracellular targets and without causing damage to the effector cell itself? To prevent unintentional reactions, either special intracellular transport routes for NO, e.g., in acidic compartments, or, alternatively, harmless NO-transport molecules must exist. Several intracellular transport forms of NO can be discussed but have not been proven to date (Table I).

After being secreted by the effector cell in a way not yet understood, NO then diffuses to a target cell located nearby and hits its plasma membrane. O_2 and NO closely resemble each other in diffusability and fluid-phase membranes are no diffusion barriers for NO (31–33). There are several targets for NO at the surface or within the plasma membrane, e.g., transport and signaling proteins and surface receptors among others. To date, reactions of NO with surface receptors leading to a chemical modification of these receptor proteins have not been reported, with the exception of the neuronal NMDA receptor–channel complex. However, the proposed mechanism, inhibition of the redox modulatory site of this complex via S-nitrosylation (34), has been recently questioned (35). Permeability to K^+, Na^+, H^+, and Ca^{2+} is the most important factor controlling the proton motive force and the membrane potential. A number of publications show activation of K^+ channels (36–38) as well as Na^+-K^+-ATPase (39) in vascular smooth muscle cells by low concentrations of NO (1–10 μM). On the other hand, high NO concentrations (0.1–1 mM) inhibit neuronal Na^+-K^+-ATPase (40), K^+ and Ca^{2+} channels in islet cells (41), and H^+-ATPase in brain synaptic vesicles (43). In addition, NO has been shown to depolarize the membrane potential of a tumor cell line (41). In conclusion, NO changes ion currents through the plasma membrane and thus alters the plasma membrane potential (for review see Ref. 44).

After diffusion into the target cell, NO can inhibit SH-dependent enzymes via S-nitrosylation (Table II). Notably, inhibition of creatine kinase, affecting local ATP regeneration, might contribute to NO-mediated cell injury. A second mechanism of NO-mediated enzyme inhibition has been found with certain heme-containing enzymes, e.g., cytochrome P450 isoenzymes (57–59). After formation of a heme–NO adduct, a secondary oxygen-dependent reaction takes place which results in an irreversible nitration of a tyrosine in the active-site pocket (60). NO also mediates Fe^{2+} release from target cells (61), destroying Fe–S clusters in enzymes, like the citric acid cycle enzyme aconitase or ferrochelatase, which catalyze the insertion of Fe^{2+} into protoporphyrin (62, 63). Other intracellular targets for NO are proteins containing zinc fingers, ring fingers, and the LIM motif, respectively. A common feature of these structures is Zn^{2+} complexed, sometimes together with histidine imidazol nitrogens, by cysteine sulfur ligands. This creates tertiary protein structural "finger" domains that specifically bind to DNA or RNA sequences (for review see Ref. 64). Many of these proteins are involved in transcription, replication,

TABLE II

Enzymes Shown to Be Inhibited by Nitric Oxide via S-nitrosylation

Enzyme	Ref.
Glyceraldehyde-3-phosphate dehydrogenase	45
Protein kinase C	46
Phosphotyrosine protein phosphatase	47
Calpain	48
Glutathione peroxidase	49
Glutathione reductase	50
Ecto-5′ nucleotidase	51
Methionine synthase	52
Creatine kinase	53, 54
Papain, bromelain (cysteine proteases)	55
Cytochrome P450 aromatase	56

recombination, or restriction. We could show that NO mediates Zn^{2+} release *in vitro* from the Zn^{2+} storage protein metallothionein and inhibits the DNA-binding activity of the zinc finger transcription factor LAC9 (65). Others found NO-mediated inhibition of protein kinase C (46), which contains a zinc finger in its regulatory domain, and of the zinc finger DNA repair enzyme Fpg (66). NO inhibits alcohol dehydrogenase, which contains both a catalytical and a structural zinc finger domain, and this inhibition is correlated with the release of Zn^{2+} (67a; K. D. Kröncke, unpublished observations). In addition to these *in vitro* studies, we recently found NO-mediated intracellular Zn^{2+} release in live cells (67b). NO has also been shown to inhibit the transcription factor NF-κB via induction and stabilization of its inhibitor IκBα (68) and additionally to inhibit the DNA-binding activity of NF-κB itself (69, 70). DNA binding of the transcription factor AP-1 is also inhibited by NO (71).

Taken together, these results strongly suggest that NO can inhibit several intracellular enzymes and profoundly affects the cellular gene transcription machinery.

3. NO AND MITOCHONDRIAL FUNCTIONS

Another intracellular target of NO are mitochondria. NO has been shown to change ion currents through the mitochondrial membrane leading to release of Ca^{2+} into the cytosol (43, 72). It is long known that activated macrophages inhibit the mitochondrial respiration of target cells (73). In the mitochondrial membrane, ATP is produced through the coupling and successive reduction of NADH to NAD^+ by oxidizing O_2 to H_2O via a gradient of enzyme redox potentials within the electron transport chain. The enzymes directly involved are complexes I, III, IV, and V (today complex II is known not to be part of this cascade but to supply electrons from the citric acid cycle). Although there are various hemes and Fe–S clusters present (more exactly, hidden) in any of these protein complexes, only the cytochrome c oxidase (complex IV) is inhibited by NO via binding to its heme moiety in a reversible manner (74–78). This is analogous to the well-studied inhibitory effects of CO and CN^-, which are isoelectronic to NO. Complexes I and III are relatively insensitive to NO (78, 79). However, $O_2^{-\bullet}$ is a by-product of the mitochondrial respiratory electron transport and its production is enhanced in the presence of electron transport chain inhibitors such as rotenone or antimycin A or CN^- (80). By analogy, (reversible) inhibition of complex IV by NO may cause a (transient) inhibition of the electron flow yielding increased $O_2^{-\bullet}$ synthesis by complexes I and III. $O_2^{-\bullet}$ and NO may then react to the strong oxidant peroxynitrite anion ($ONOO^-$) which has been shown to irreversibly inhibit complexes I, II, and III (77,78, 81) but not complex IV (76, 78). In conclusion, the current hypothesis is that a reversible NO-mediated inhibition of the respiratory chain may result in enhanced intracellular $O_2^{-\bullet}$ and subsequent $ONOO^-$ production (Fig. 2). This may lead to peroxidation of lipids, to destruction of Fe–S clusters within enzymes, and to inhibition of the mitochondrial manganese superoxide dismutase via nitration (82) and may furthermore induce Ca^{2+} efflux from the mitochondria (83). This altogether causes irreversible damage to the power stations of the cell.

4. NO AND THE NUCLEUS

The nucleus is a further cellular target for NO. NO has been shown to cause G:C → A:T transitions and to mediate DNA strand breaks (Table III), both suggested to be the results of N-nitrosylation of deoxynucleotides, thus yielding deaminated DNA bases (for review see Ref. 96). However, indirect induction of DNA strand breaks, e.g., via intracellular oxygen radical and/or peroxynitrite anion generation (see above), via N-nitrosamine formation and subsequent alkylation reactions, via activation or inhibition of enzymes necessary for nuclear homeostasis, or via other mechanisms, cannot be excluded to date. Indeed, NO has been shown to induce oxidative DNA damage in an activated macrophage cell line (97) and to inhibit enzymes involved in DNA repair (Table III). Because DNA damage is a constant hazard in natural environments induced by chemicals, ionizing radiation, or UV light, leading to a variety of biological consequences such as mutation induction, blocking of transcription, and replication, cells have evolved an array of mechanisms for repair. Recent

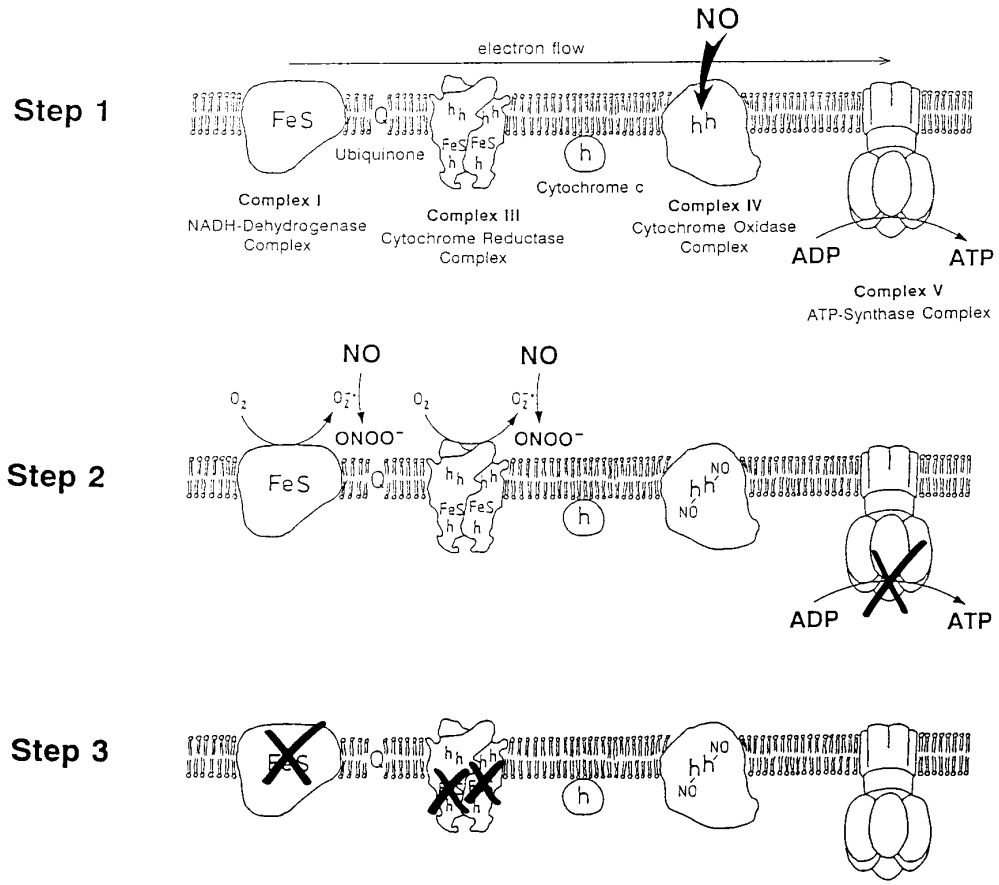

FIG. 2. Effects of NO toward the mitochondrial respiratory chain. Step 1: NO inhibits O_2 consumption by competing with oxygen for the heme (h) binding site of complex IV. Inhibition of this enzyme complex by NO inhibits the electron flow and therefore oxidative phosphorylation, but as the inhibition is reversible, this is presumably not lethal for cells. Step 2: inhibition of complex IV potentially increases the concentration of $O_2^{-\bullet}$ as electrons leak from reduced electron transport centers. NO and $O_2^{-\bullet}$ may react to the strong oxidant peroxynitrite anion ($ONOO^-$). Step 3: peroxynitrite anions may destroy Fe–S clusters of the complexes I–III and may induce lipid peroxidation thus causing irreversible damage of mitochondria.

observations suggest that damaged DNA is processed not only by DNA repair enzymes but also by other nuclear factors involved in a variety of cellular functions. Most forms of DNA alterations are recognized by DNA excision repair pathways catalyzing removal of damaged or modified regions. Thus, strand breaks induced by endonucleases at active repair sites serve to signal the presence of DNA damage, which is then repaired by polymerization and ligation. Proliferating cells are especially vulnerable to DNA damage due to the added demands of cellular growth and division. Delaying progression through the cell cycle at so called "cell-cycle checkpoints" provides the time necessary for repair. In addition to cell-cycle arrest, DNA damage can induce apoptosis (programmed cell death) in cells of multicellular organisms, thus eliminating cells in which damage is beyond repair possibilities, thereby preventing propagation of mutations (see below). Initiation of either cell-cycle arrest or apoptosis requires induction of the tumor-suppresser protein p53, the main biological function of which is to be a "guardian of the genome" (for reviews see Refs. 98 and 99). Another important protein involved in DNA repair is the poly(ADP-ribose)polymerase (PARP). The PARP is a constitutively expressed nuclear protein (approximately 10^6 copies/nucleus) which is regarded as a molecular nick sensor and has a functional role dur-

TABLE III

Effects of NO toward the Nucleus

Effect of NO	Ref.
DNA damage	
G:C → A:T transitions	
Yeast	26
Human kidney cell line	84, 85
DNA strand breaks	
Human lymphoblast cell line	27
Rat islet cells	86
p53 expression	
Mouse macrophage cell line, rat islet cell line	87
Mouse thymocytes	88
Human fibroblasts	89
Human colon adenocarcinoma cell line	90
Activation of poly(ADP-ribose)polymerase (PARP)	
Rat neurons	91
Rat islet cells	92
Inhibition of DNA repair enzymes	
Ribonucleotide reductase	93, 94
O^6-Methylguanine–DNA methyltransferase	95
Formamidopyrimidine–DNA glycolase (Fpg)	66

ing rejoining of DNA strand breaks (for reviews see Refs. 100 and 101). Following its binding to DNA breaks, PARP automodifies itself by adding several branched polymer chains of up to 200 ADP-ribose residues each resulting in PARP inhibition and causing its dissociation from the DNA strand breaks. The poly(ADP-ribose)polymers synthesized in response to DNA damage are then degraded within 1–2 min by specific glycohydrolases. The physiological role of the PARP is not exactly known to date. It either protects DNA strand breaks during early stages of recombination and repair or it may transiently block DNA replication, thus inducing a cell-cycle arrest and providing time or space for assembly of the DNA repair complex. It may also simply constitute an emergency signal. Whatsoever the exact roles of p53 and PARP are, induction of p53 protein expression and activation of PARP serve as an indirect indicator for DNA damage. NO treatment has been shown to induce p53 expression and to activate PARP in neurons and in islet cells (see Table III). While p53 expression per se is not detrimental for cells, activation and subsequent poly(ADP-ribosylation) of PARP lead to a severe cellular depletion of ATP and NAD^+. Consequently, PARP inhibitors have been shown to partially protect islet cells (102) and neurons (91) from NO-mediated cell death and to inhibit NO-mediated cellular NAD^+ depletion in islet cells (92). In addition, NO treatment of islet cells isolated from mice with a disrupted PARP gene did not result in NAD^+ depletion and, hence, these cells exhibited an increased resistance toward NO (103).

In conclusion, NO mediates DNA damage, thereby causing depletion of cellular ATP and NAD^+ levels which may contribute to cell lysis (Fig. 3).

5. CELL SUSCEPTIBILITIES AND DEFENSE MECHANISMS TOWARD NO

Large differences were found in the sensitivities toward the cytotoxic action of NO of different mammalian cell types and also of various tumor cell lines (20). While islet cells (104) and neurons (105, 106) are extremely sensitive toward NO secreted by activated macrophages or microglia, as well as NO generated by NO donors (20, 107), other cell types apparently are much less sensitive. Figure 4 shows that 1 mM NO-donor SNAP is completely toxic for rat islet cells within 16 h, but that human keratinocytes or rat aorta endothelial cells are hardly affected. Rat hepatocytes exhibit an intermediate sensitivity toward NO. Regarding the two cell lines P815 mastocytoma cells and L929 fibroblasts, the former are very sensitive toward NO and the latter are totally resistant, even at high concentrations. To date, exact data are lacking to explain these different sensitivities toward NO. Different cellular capacities to scavenge or to detoxify NO exist, but also the capacity to rapidly switch from aerobic to anaerobic respiration in promoting glycolysis, as shown for Ehrlich ascites tumor cells (108), and/or effective DNA or other cellular repair mechanisms may play a role. Glutathione is probably the most important cellular antioxidant and it has been found that NO reacts with intracellular glutathione yielding *S*-nitrosoglutathione (109). In addition, glutathione depletion has been found to dramatically increase cellular sensitivity toward NO, suggesting that intracellular glutathione pools act to scavenge NO or NO-derived species (110). However, the intracellular thioredoxin system (111) and glutathione peroxidase (112) mediate rapid NO release from *S*-nitrosoglutathione. Most probably it is not the absolute cellular

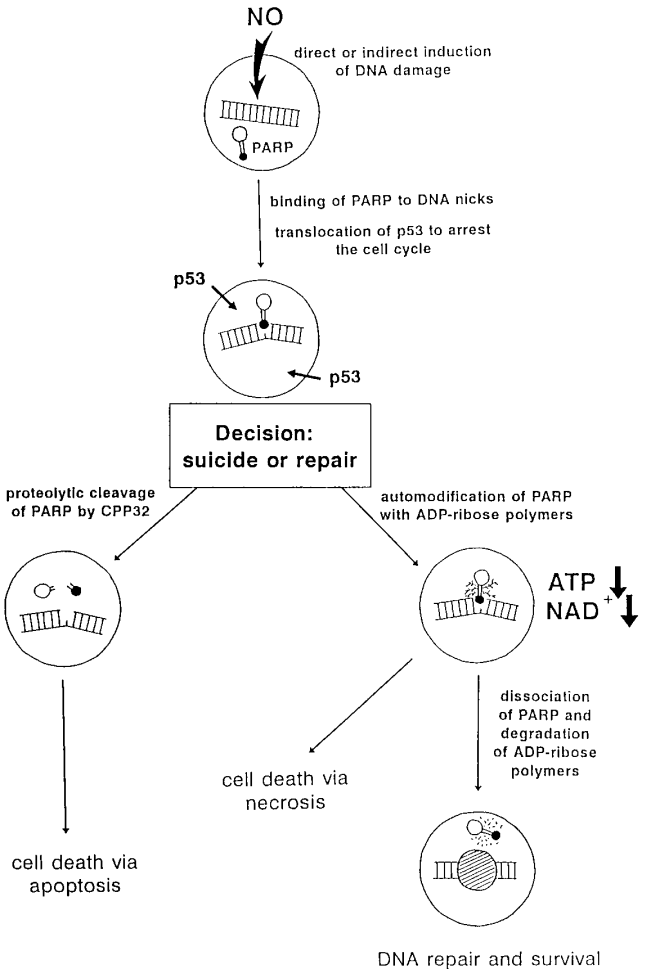

FIG. 3. Interactions of NO with nuclear DNA. NO directly or indirectly induces DNA strand breaks leading to DNA nick-binding of the constitutively expressed nuclear enzyme poly(ADP)ribose-polymerase (PARP). Simultaneously, p53 is induced and translocated into the nucleus to arrest the cell cycle to allow DNA repair. At this stage, it is decided whether the cell tries to repair its damaged DNA or whether the damage is too great to be repaired. If the latter is the case, a cascade of cysteine proteases is activated leading to cleavage and inactivation of PARP by CPP32 resulting in cell death via self-destruction (apoptosis). If the cell decides to try a DNA repair, PARP automodifies itself via poly(ADP)ribosylation. This modification leads to dissociation of PARP from the DNA nicks and to degradation of the ADP-ribose polymers. The DNA repair machinery then takes over to restore the damaged DNA. This whole process is very energy- and substrate-consuming, causing a rapid and severe depletion of cellular ATP and NAD^+, which may lead and/or contribute to cell death via necrosis.

glutathione concentration, but differences in the activity of the whole cellular antioxidant system, consisting of catalase, superoxide dismutases, glutathione reductase, glutathione peroxidase, thioredoxin, thioredoxin reductase, and/or others and the cellular capacity to supply reduction equivalents, e.g., NADPH via the hexose monophosphate shunt (109), which may lead to survival or cell death after nitrosative stress.

The search for reasons to explain the varying cellular NO resistances leads to the question of whether inducible cellular defense mechanisms against NO may have evolved. NO-induced expression of defense molecules was first detected in bacteria, where low concentrations of NO induce the expression of SoxR-regulated genes (113). The products of these genes include Mn-SOD, endonuclease IV (a DNA repair enzyme for oxidative damage), glucose-6-phosphate dehydrogenase (to maintain intracellular NADPH levels to fuel antioxidant enzymes), and others and are regarded as oxidative stress responses. Activation of these genes confers bacterial resistance toward NO secreted by activated macrophages (113, 114). The NO-donor *S*-nitrosocysteine S-nitrosylates and activates the *Escherichia coli* transcription factor OxyR, which controls genes also involved in the oxidative stress response like catalase, glutathione reductase, and alkyl hydroperoxidase reductase (115). Similar inducible defense reactions have also

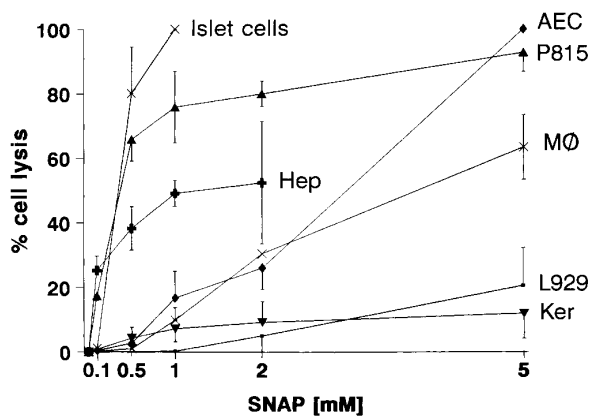

FIG. 4. Cell susceptibilities toward NO. Cells were cultured with various concentrations of the NO-donor SNAP. After 16 h, cell viability was determined by trypan blue exclusion. Islet cells and the tumor cell line P815 were the most sensitive cells studied, while keratinocytes and the tumor cell line L929 were completely resistant toward NO generated by SNAP. Data are taken from Ref. 20 or, in the case of the keratinocytes, are unpublished (V. Schui and K. D. Kröncke).

been detected in mammalian cells. Heat shock as well as the overexpression of the major heat shock protein hsp70 induced resistance to NO in islet cells (116, 117). Moreover, an NO donor has been shown to induce hsp70 expression in a hepatoblastoma cell line as well as in organs of animals (118). Pretreatment of hepatocytes with low concentrations of the NO-donor SNAP induced expression of ferritin and conferred partial resistance toward subsequent treatment with high NO-donor concentrations (119). Furthermore, pretreatment of the macrophage-like cell line RAW 264.7 with lipopolysaccharide plus IFN-γ and simultaneous inhibition of NO synthesis as well as pretreatment with low nonlethal NO-donor concentrations mediated protection upon subsequent apoptotic cell death induced by a high NO-donor concentration (120). NO donors have also been shown to induce the expression of Mn-SOD mRNA in vascular smooth vessel cells (121) and to induce the stress proteins heme oxygenase in aorta endothelial cells (122), stress-activated protein kinases in endothelial and mesangial cells (123), and the C-reactive protein in islet cells (K. Fehsel, unpublished work). A cell line overexpressing metallothionein was found to be more resistant to the cytotoxic NO effect (124). Transfection of the protooncogene bcl-2 delayed NO-induced apoptosis in the macrophage-like cell line RAW 264.7 (125) and partially protected P815 tumor cells from NO-mediated lysis (126). All of these results strongly suggest that several constitutive and/or inducible defense systems exist in mammalian cells that may neutralize the damaging effects of NO and that differential expression of defense reactions may account for the varying cellular susceptibilities.

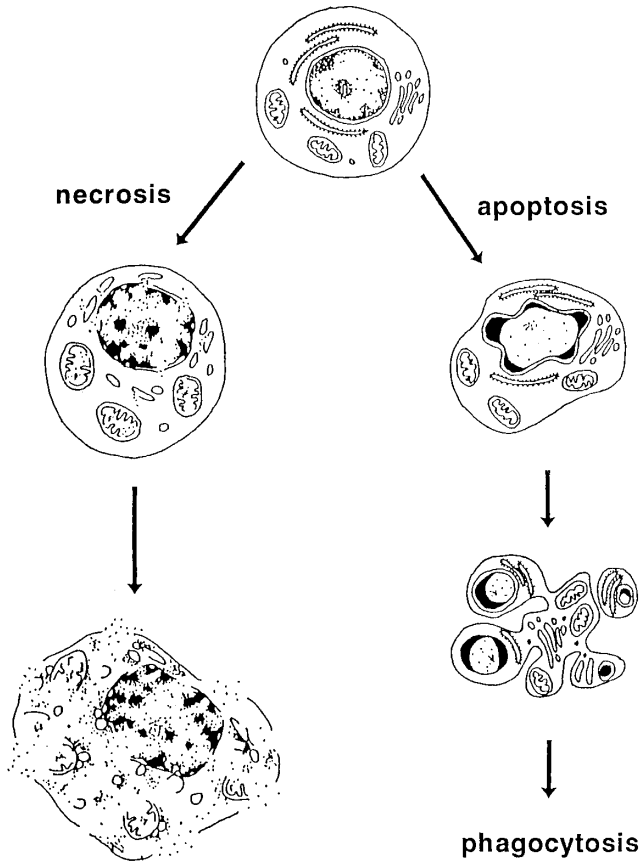

FIG. 5. Ultrastructural cellular events during necrosis or apoptosis (programmed cell death). Necrotic cell death is characterized morphologically by condensation of chromatin without radical change in its distribution, by cell and organelle swelling, and by subsequent cell membrane breakdown and leakage of cell content. Apoptotic cell death is characterized by compaction of chromatin against the nuclear membrane, cell shrinkage with preservation of organelles, and most often nuclear and cytoplasmic budding to form membrane-bound fragments known as apoptotic bodies, which are rapidly phagocytosed by adjacent parenchymal cells or macrophages (according to Ref. 127).

6. NO-MEDIATED CELL DEATH VIA APOPTOSIS OR NECROSIS

Cells can die via two pathways, necrosis or apoptosis (programmed cell death). Necrosis is a pathological form of cell death caused by physical, chemical, or osmotic damage with consecutive disruption of internal and external membranes, leading to cell swelling and lysis with release of cytoplasmic material (Fig. 5). This will often trigger an inflammatory response. In contrast, apoptosis is an innate cellular program of cell death which can be regarded as the opposite of proliferation. Most, if not all, animal cells have the ability to activate this suicide program when they are no longer needed or have become seriously injured. During apoptosis, the nucleus and the cytoplasm are condensed and fragmented yielding the so-called apoptotic bodies (Fig. 5) which are rapidly phagocytosed by macrophages or neighboring cells. This orderly packaging and removal prevents presentation of cytoplasmic cell con-

TABLE IV

NO-Induced Death of Primary Cells via Apoptosis or Necrosis

Apoptosis	Ref.	Necrosis	Ref.
Mouse activated macrophages	134, 135	Rat islet cells	104, 86
Human chrondrocytes	136	Rat oligodendrocytes	141
Rat neurons	137	Rat neurons	107, 137
Mouse thymocytes	88		
Rat vascular smooth muscle cells	138		
Mouse dendritic cells	139		

tent by antigen-presenting cells, thus avoiding subsequent inflammatory and/or autoimmune reactions. This form of physiological cell death is used during embryogenesis, for elimination of autoreactive lymphocytes, for homeostasis, as a defense mechanism, and in aging (for reviews see Refs. 128 and 129).

Initially, nuclear DNA degradation ("DNA ladder") has been regarded as a hallmark of apoptotic cell death (for review see Ref. 130). However, recent investigations show that a cytoplasmic proteolytic cascade is essential and in addition that interleukin-converting enzyme-like cysteine proteases like CPP32 play a key role in apoptosis (for review see Ref. 131). CPP32 cleaves and thereby inactivates PARP (132, 133), which is an enzyme involved in rejoining DNA during repair (see Fig. 3). Thus, cellular activity of PARP to repair DNA damage and realization of the apoptotic pathway to degrade DNA exclude each other.

The question arises whether NO-mediated cell death occurs via necrosis or via apoptosis. The answer is both (Table IV). In macrophages, chondrocytes, thymocytes, smooth muscle cells, and dendritic cells, cell activation induced NO synthesis or NO-donor treatment mediated subsequent cell death bearing all morphological characteristics of apoptosis. In contrast, islet cells and oligodendrocytes after NO treatment show cell lysis as it is typical for necrosis with no morphological evidence of condensed nuclei or fragmentation (20, 140). Because NO-mediated lysis of islet cells cannot be inhibited or delayed by compounds that usually inhibit apoptosis (86) and because NO mediates activation of PARP in islet cells (92), these cells apparently die via necrosis despite early occurrence of DNA strand breaks. In the case of neurons, the situation is less clear, as apoptotic as well as necrotic cell death is induced by NO (107, 137). Additionally, PARP activation has been demonstrated (91). Thus, neurons exposed to NO probably die via both pathways. NO has also been shown to induce apoptosis *in vitro* in several cell lines, but as apoptosis is the opposite of proliferation, results obtained with rapidly proliferating tumor cell lines may not reflect NO-mediated death mechanisms of nontransformed cells.

In conclusion, in susceptible cells NO can induce apoptotic as well as necrotic cell death, depending on the cell type investigated, and to date it seems not to be predictable which cell type dies via which mechanism.

7. PROTECTIVE FUNCTIONS OF NO?

Despite the manyfold cytotoxic interactions described above, several reports convincingly demonstrate a protective role for NO in oxidative stress. NO inhibits lipid peroxidation by ferrous compounds/H_2O_2 (141), by reactive oxygen intermediates (142), by Fe^{2+} (143), or by azo compounds (144). NO also inhibits lipid peroxidation of LDL by activated macrophages (145) or catalyzed by Cu^{2+} (146, 147). Low concentrations of NO have been shown to protect cells from short-term treatment with H_2O_2 (148, 149) or with alkyl peroxide (150) and to prevent oxidized LDL- or H_2O_2-mediated endothelial cell injury (151, 152). In addition, inhalation of NO significantly increased survival of rats exposed to hyperoxia (153). Intranigral coinfusion of NO with ferrous citrate also exhibited protective effects compared to infusion of the latter only (154). It is concluded that NO acts

as a chain-breaking antioxidant to scavenge peroxyl radicals (for reviews see Refs. 155 and 156).

Protective effects of NO have also been reported during cerebral and myocardial ischemia and/or reperfusion. These effects of NO are most probably indirect effects of NO as a consequence of its vasodilatory activity to increase the blood flow, its capability to inhibit adhesion of lymphocytes, monocytes, and neutrophils to the endothelium (thereby decreasing local $O_2^{-\bullet}$ generation), and its ability to inhibit platelet aggregation, thus decreasing capillary occlusions (for reviews see Refs. 157 and 158). During reperfusion of ischemic areas, a burst of reactive oxygen intermediates, especially $O_2^{-\bullet}$, is generated. NO can then either react with $O_2^{-\bullet}$ to the peroxynitrite anion-enhancing toxicity (for review see Ref. 159) or scavenge reactive peroxyl radicals as described above. Due to these opposing effects it is still an open question as to whether direct effects of NO are protective or damaging. In brain the situation is even more complex. Production of NO during cerebral ischemia has both neuroprotective and neurotoxic effects. Vascular NO production protects against ischemic brain injury, while neuronal NO production may lead to neurotoxicity. NO production by iNOS is involved in later stages of infarctions elicited by focal ischemia and probably plays an important role in late development of tissue damage (for reviews see Refs. 160 and 161).

In conclusion, NO may be cytoprotective by directly acting as a potent terminator of radical propagation systems or as inductor of defense responses. However, higher concentrations of NO most probably exhibit toxic effects.

CONCLUSIONS

Although exhibiting direct protective effects as chain-breaking antioxidant, NO (and/or oxidized intermediates) at high concentrations affects many cellular functions simultaneously. NO changes ion currents through membranes, inhibits the cellular respiration and various SH-dependent enzyme activities, mediates DNA damage, and affects the cellular transcription machinery. In susceptible cells these multiple effects may lead to cellular necrosis or apoptosis depending on the cell type or on local NO concentrations. Although knowledge about cytotoxic effects of NO is steadily increasing, we are still at the beginning of understanding as to how, why, when, and where cells are killed by NO.

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