NO Inhibits Cytokine-Induced iNOS Expression and NF-κB Activation by Interfering With Phosphorylation and Degradation of IκB-α

Abstract—Nitric oxide (NO) is known to have antiatherogenic and anti-inflammatory properties, but its effects on the cytokine-induced nuclear factor-kappa B (NF-κB) activation pathway in relation to the regulation of inducible nitric oxide synthase (iNOS) gene in vascular smooth muscle cells (VSMCs) remain elusive. To elucidate the roles of NO in the regulation of cytokine-induced NF-κB activation and consequent iNOS gene expression, we studied the effects of NO donors [[(±)-(E)-ethyl-2-[(E)-hydroxyamino]5-nitro-3-hexeneamide (NOR3) and sodium nitroprusside] on interleukin (IL)-1β-induced NF-κB activation and IκB-α degradation and subsequent iNOS expression in rat VSMCs. Northern blot and Western blot analyses demonstrated that NO donors decreased IL-1β-induced IκB-α degradation and protein expression. Electrophoretic mobility shift assay using synthetic oligonucleotide corresponding to the downstream NF-κB site of rat iNOS promoter as a probe showed that NOR3 inhibited IL-1β-induced NF-κB activation and its nuclear translocation, as demonstrated with immunocytochemical study. These effects were independent of guanylate cyclase activation; an inhibitor of soluble guanylate cyclase (1H-oxadiazolo-1,2,4-[4,3-α]quinoxaline-1-one) had no effect on NOR3-induced inhibition of NF-κB activation or iNOS mRNA expression by IL-1β, and a cGMP derivative (8-bromo-cGMP) failed to mimic the effects of NO donors. Western blot analysis using anti–IκB-α and anti–phospho-IκB-α antibodies revealed that IL-1β induced a transient degradation of IκB-α preceded by a rapid appearance of phosphorylated IκB-α, both of which were completely blocked by NOR3. A proteasome inhibitor (MG115) blocked IL-1β-induced transient degradation of IκB-α and stabilized the appearance of phosphorylated IκB-α stimulated by IL-1β. NOR3 inhibited the appearance of IL-1β-induced phosphorylated IκB-α even in the presence of MG115. Our results indicate that an inhibitory action by NO on cytokine-induced NF-κB activation and iNOS gene expression is due to its direct blockade on phosphorylation and subsequent degradation of IκB-α via the cGMP-independent pathway in rat VSMCs. (Arterioscler Thromb Vasc Biol. 1998;18:1796-1802.)

Key Words: NF-κB  ■  IL-1β  ■  inducible nitric oxide synthase  ■  IκB-α

Nitric oxide (NO) is synthesized from L-arginine by NO synthases (NOSs). Three distinct isozymes of NO have been identified to date: 2 Ca2+/calmodulin-dependent constitutive isozymes dominantly expressed in the brain and endothelium, and a Ca2+-independent, cytokine-inducible isozyme (iNOS).1 iNOS produces large amounts of NO in response to bacterial lipopolysaccharides (LPS) and certain cytokines in a variety of cells, including vascular smooth muscle cells (VSMCs).2 NO possesses diverse physiological properties, such as vasodilatation, neurotransmission, and mediation of immune responses.1 High-output NO produced by iNOS in VSMCs not only causes inhibition of cell proliferation but apoptosis of VSMCs as well.3,4 Therefore, regulation of iNOS gene expression has been implicated in the pathogenesis of vascular remodeling and atherosclerosis.5 Many of the biological effects of NO have been attributed to cGMP generation via the stimulation of soluble guanylate cyclase, although a cGMP-independent mechanism is also involved in its diverse actions.

The promoter region of the rat and mouse iNOS gene contains several potential cis-elements for the binding of different transcription factors, among which 2 putative binding sites for nuclear factor-kappa B (NF-κB) exist in the upstream (GGGGATTTTCC, nucleotides −965 to −955; NF-κB1) and downstream (GGGGACTCTCC, nucleotides −107 to −97; NF-κBd) regions.6–8 The sequence of NF-κBd is unique in that it is found only in murine and human iNOS genes. It has been shown that a key region of the promoter activity in mediation of LPS inducibility resides in the NF-κBd region in mouse macrophages.5,7 However, its role in mediation of iNOS expression in response to cytokines in VSMCs remains largely unknown.

NF-κB complexes function as a pleiotropic regulator of many genes modulating immunologic and inflammatory pro-
cesses. NF-κB contains heterodimeric complexes, usually consisting of p50 and p65 (Rel-A) subunits and p50/p50 homodimers in human VSMCs.5 p50/p65 heterodimer associates with its cytoplasmic inhibitor, IκB-α, to form an inactive cytoplasmic ternary complex. p65 subunit may also complex with p105, a precursor protein of p50, as an inactive form. Activation of NF-κB by LPS or cytokines requires either degradation of IκB-α10 or proteolytic cleavage of p105 through a common ubiquitin-proteasome pathway after phosphorylation.11 After degradation of IκB-α, an active NF-κB translocates to the nucleus and expresses the activation of a plethora of genes. Recently, a cytokine-responsive IκB-α kinase (IKK) that activates NF-κB by phosphorylation of Ser27 and Ser36 residue in IκB-α has been identified.12–16 It has been reported that NO inhibits NF-κB by induction and stabilization of IκB-α in human endothelial cells.17 However, it remains unknown whether NO inhibits cytokine-induced NF-κB activation via inhibition of phosphorylation and degradation of IκB-α, thereby blocking iNOS gene expression in VSMCs.

These observations led us to examine (1) whether interleukin (IL)-1β–induced IκB-α gene expression is mediated by the NF-κB activation pathway involving phosphorylation and subsequent degradation of IκB-α in cultured rat VSMCs and (2) whether NO inhibits IL-1β–induced IκB-α gene expression by interfering with phosphorylation and subsequent degradation of IκB-α.

Methods

Materials

Human recombinant IL-1β was kindly provided by Otsuka Pharmaceutical. Murine recombinant tumor necrosis factor (TNF)-α was purchased from Gibco BRL; (2S,−)-ethyl-2-[(1S)-hydroxyamino]-5-nitro-3-hexeneamide (NOR3), SDS, and EDTA were purchased from Wako Pure Chemical; 8-bromo-cGMP, N-acetylcysteine, 1H-oxadiazolo[1,2-a]quinoxaline-1-one (ODQ), PMSF, DTT, poly(dI-dC), and EGTA were purchased from Sigma Chemical; pyrrolidinedithiocarbamate was purchased from Katayama Chemical; sodium nitroprusside (SNP) was purchased from Calbiochem Novabiochem; MG115 was purchased from Peptide Institute; [α-32P]dCTP was purchased from Amersham International; and 8-bromo-cGMP, [3H]adenosine, [3H]cAMP, and [3H]norepinephrine were from The Radiochemical Centre (Amersham). The cDNA probe for rat iNOS recently cloned from rat vascular endothelial cells20 was labeled with [α-32P]dCTP (111 TBq/mmol) by random-primed labeling method. RNA immobilized on the membrane was hybridized with the labeled probes, washed in 0.1×SSPE/0.5% SDS, and autoradiographed.

Cell Culture

VSMCs from the thoracic aorta of 15-week-old male Wistar rats were prepared by the explant method and cultured in Dulbecco’s modified Eagle’s medium containing 10% FBS at 37°C in a humidified atmosphere of 95% air and 5% CO2, as described previously.18 Subcultured VSMCs (15th to 20th passages) from 2 independent isolates were used in the experiments.

Electrophoretic Mobility Shift Assay (EMSA)

Confluent VSMCs (5×105 cells per dish) pretreated with or without NO donors (NOR3, SNP) or MG115 for 60 minutes were stimulated with IL-1β for 6 hours, and total RNAs were extracted by the acid guanidinium thiocyanate-phenol-chloroform methods.19 Total RNAs (20 μg), separated by formaldehyde/1.1% agarose gel electrophoresis, were transferred to a Magna Graph nylon membrane (Micron Separations Inc). The cDNA probe for rat iNOS recently cloned from rat endothelial cells20 was labeled with [α-32P]dCTP (111 TBq/mmol) by random-primed labeling method. RNA immobilized on the membrane was hybridized with the labeled probes, washed in 0.1×SSPE/0.5% SDS, and autoradiographed.

Western Blot Analysis

Western blot analyses were performed essentially as described.21 Confluent cells (5×105 cells per dish), pretreated with or without NO donors (NOR3, SNP) or MG115 for 60 minutes, were stimulated with IL-1β for the indicated times for IκB-α and phospho-IκB-α, or for 15 hours for iNOS. Cells were lysed in 50 mmol/L Tris-HCl, pH 6.8 (10% glycerol, 1% SDS, 1 μg/mL pepstatin, 2 μg/mL leupeptin, 2 μg/mL aprotinin, and 1 mmol/L PMSF). Whole-cell lysates were boiled, and extracted proteins were separated on 12% gel, for IκB-α and phospho-IκB-α or 7.5% (for iNOS) SDS-polyacrylamide gel and transferred to Hybond ECL nitrocellulose membranes (Amersham), which were incubated overnight with rabbit polyclonal antibody for human IκB-α (1:500; Santa Cruz Biotechnology), rabbit polyclonal antibody for human phospho-IκB-α (Ser32) (1:1000; New England Biolabs), or mouse monoclonal antibody for murine iNOS (1:1000; Transduction Laboratories) at 4°C. Anti–phospho-IκB-α antibody detects IκB-α only when phosphorylated at Ser32 and has no cross-reactivities with the corresponding phosphorylated Ser of IκB-β or IκB-ε. After extensive washing, the secondary antibody (donkey anti-rabbit IgG or sheep anti-mouse IgG horseradish peroxidase; 1:500, Amersham) was applied for 1 hour, and exposure was performed by using an ECL kit (Amersham).

Immunoochemical Staining

Subconfluent cells grown on LAB-TEK Chamber Slide (Nalge Nunc Int) were treated with IL-1β in the absence or presence of NOR3 for 2 hours, fixed with 70% acetone for 20 minutes at room temperature, and then washed with PBS for 10 minutes. Goat polyclonal antibody specific for NF-κB p50 subunit (Santa Cruz Biotechnology) was used; the antibody did not show any cross-reactivities with p105, p52, or p100. Immunostaining was visualized with the indirect immunoperoxidase avidin-biotin-peroxidase kit (Vector Laboratory).

Results

NF-κB Activation by IL-1β

To determine whether NF-κB activation was induced with IL-1β in rat VSMCs, EMSA was performed using synthetic oligonucleotides corresponding to the NF-κB binding site (−107 to −57).
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Kribosomal RNA (middle panel), and 2 hours for EMSA of NF-

–97) of the rat iNOS promoter as a probe (Figure 1). IL-1β (10 ng/mL) caused a distinct shifted band, whereas there was no distinct band in the control cells. The specificity of the NF-κB binding gel shift assay was examined using coincubation with excess unlabeled probe as a competitor. The band was completely eliminated in the presence of a 100-fold molar excess of unlabeled oligomers (Figure 1A). For characterization of NF-κB subunits, specific antibodies for human p50 and p65 subunits were examined. The IL-1β–induced NF-κB protein-DNA complexes were supershifted by anti-p50 antibody, whereas anti-p65 antibody caused a supershifted band in addition to the reduced shifted band (Figure 1B).

NO Inhibits IL-1β–Induced NF-κB Activation and iNOS mRNA and Protein Expression

We studied whether NO donors affect NF-κB activation and iNOS expression induced by IL-1β in rat VSMCs (Figure 2).

Northern blot analysis using rat iNOS cDNA as a probe revealed that IL-1β (10 ng/mL) induced iNOS mRNA expression (4.5 kb), the effects of which were dose-dependently (10−3 to 10−5 mol/L) suppressed by NOR3 (Figure 2A; top panel). EMSA showed that NOR3 similarly blocked the IL-1β–induced NF-κB activation in a dose-dependent manner (10−5 to 10−3 mol/L) (Figure 2A; bottom panel). NOR3 (10−3 mol/L) completely blocked both NF-κB activation and iNOS mRNA expression stimulated by IL-1β. SNP (10−3 mol/L) also attenuated the IL-1β–induced iNOS mRNA expression and NF-κB activation (Figure 2B).

To determine whether the inhibitory effect of NO donors is mediated via a cGMP-dependent mechanism, the effects of a selective inhibitor of soluble guanylate cyclase (ODQ) and a cell-permeable cGMP analogue (8-bromo-cGMP) were tested (Figure 3). ODQ (10−5 mol/L) did not affect the inhibitory effect of NOR3 (10−4 mol/L) on IL-1β–induced iNOS mRNA expression or NF-κB activation (Figure 3A). 8-Bromo-cGMP (10−3 mol/L) did not inhibit the IL-1β–induced iNOS mRNA expression or NF-κB activation (Figure 3B). Western blot analysis using specific anti-murine iNOS antibody demonstrated a distinct band of 130-kDa iNOS protein after stimulation with IL-1β (10 ng/mL), which was completely abolished in the presence of NOR3 (10−4 mol/L) (Figure 4). These data indicate that NO blocks IL-1β–induced NF-κB activation as well as iNOS mRNA and protein expression via a cGMP-independent mechanism.

NO Prevents IL-1β–Induced NF-κB Nuclear Translocation

To confirm that NO donor prevents nuclear translocation of active NF-κB in rat VSMCs after cytokine stimulation, immunohistochemical staining using anti-p50 antibody was performed. Nonstimulated cells revealed a diffuse but faint distribution of immunoreactive p50 within the cytoplasm (Figure 5A). In contrast, exposure of rat VSMCs to IL-1β (10
ng/mL) resulted in dense accumulations of immunoreactive p50 within the nucleus (Figure 5B), the effect of which was prevented by pretreatment with NOR3 (10^{-3} mol/L) (Figure 5D); NOR3 added alone was without effect (Figure 5C).

**NO Prevents IL-1β–Induced IκB-α Degradation**

To determine whether IL-1β causes IκB-α degradation in rat VSMCs, Western blot analysis using anti–IκB-α antibody was performed. Addition of IL-1β (10 ng/mL) resulted in a rapid (within 15 to 30 minutes) decrease in IκB-α protein, which then returned to baseline levels within 1 to 2 hours (Figure 6A). Pretreatment with NOR3 (10^{-3} mol/L) completely prevented the IL-1β–induced transient decrease in IκB-α levels (Figure 6B); NOR3 added alone was without effect. A proteasome inhibitor, MG115 (10^{-5} mol/L), also prevented the transient decrease in IκB-α levels induced by IL-1β (Figure 6B). These data suggest that both NO and MG115 interfere with the transient degradation of IκB-α induced by IL-1β in rat VSMCs.

**NO, but Not Proteasome Inhibitor, Prevents IL-1β–Induced IκB-α Phosphorylation**

To determine whether IL-1β causes IκB-α phosphorylation in rat VSMCs, Western blot analysis using anti–phospho-IκB-α antibody was performed. Addition of IL-1β (10 ng/mL) resulted in a rapid (within 3 to 5 minutes) appearance of phosphorylated IκB-α, which peaked at 5 minutes and then decreased by 30 minutes (Figure 7A; top panel). Pretreatment with MG115 (10^{-5} mol/L) stabilized the phosphorylation of IκB-α.

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**Tables and Figures**

- **Figure 4.** Effect of NO donor on IL-1β-induced iNOS protein expression in rat VSMCs. Confluent cells pretreated without (−) or with (+) NOR3 (10^{-4} mol/L) were stimulated with IL-1β (10 ng/mL) for 15 hours and subjected to Western blot analysis using murine iNOS antibody. An arrow denotes 130-kDa iNOS protein.

- **Figure 5.** Effect of NO donor on IL-1β–induced nuclear translocation of NF-κB by immunohistochemical staining. Cells pretreated with or without NOR3 (10^{-3} mol/L) were stimulated with IL-1β (10 ng/mL) for 2 hours, fixed with 70% acetone, and stained with anti-human p50 subunit antibody. A, Control; B, IL-1β; C, NOR3; D, IL-1β + NOR3.

- **Figure 6.** Effects of NO and proteasome inhibitor on degradation of IκB-α by IL-1β in rat VSMCs. A, Confluent cells were incubated with IL-1β (10 ng/mL) for the indicated times. B, Cells were pretreated with (+) or without (−) NOR3 (10^{-3} mol/L) and/or MG115 (10^{-5} mol/L) for 60 minutes and stimulated with or without IL-1β (10 ng/mL) for 15 minutes. The extracted cell lysates were subjected to Western blot analysis using anti–IκB-α antibody.

- **Figure 7.** Effects of NO donor and proteasome inhibitor on phosphorylation of IκB-α by IL-1β in rat VSMCs. A, Confluent cells were incubated with IL-1β (10 ng/mL) pretreated without (top panel) or with (bottom panel) MG115 (10^{-5} mol/L) for the indicated times. B, Cells were pretreated with (+) or without (−) NOR3 (10^{-3} mol/L) and/or MG115 (10^{-5} mol/L) for 60 minutes and stimulated with or without IL-1β (10 ng/mL) for 5 minutes. The cell lysates were subjected to Western blot analysis using anti–phospho-IκB-α antibody.
IL-1β-induced phosphorylated IκB-α during a 30-minute incubation period (Figure 7A; bottom panel). NOR3 (10^{-3} mol/L) prevented the IL-1β-stimulated transient increase in phosphorylated IκB-α with or without pretreatment with MG115 (Figure 7B); NOR3 or MG115 added alone was without effect. These data suggest that NO inhibits rapid phosphorylation of Ser^{32} of IκB-α induced by IL-1β before its degradation.

**Discussion**

The present study clearly demonstrates that IL-1β-induced iNOS mRNA and protein expression is associated with NF-κB activation in rat VSMCs. By EMSA using a synthetic oligomer corresponding to the unique downstream NF-κB site of rat iNOS promoter as a probe, we have shown that the IL-1β specifically activated NF-κB composed of p50/p65 heterodimer that is immunologically similar, if not identical, to its human counterpart. These results are in agreement with a recent report demonstrating that NF-κB complexes were mainly composed of p50/p65 heterodimers in human cultured VSMCs.\(^9\)

Transcriptional activity of p50/p65 heterodimer of NF-κB can be regulated by at least 2 pathways.\(^11\) The p50/p65 heterodimer constitutes an inactive cytoplasmic ternary complex with the inhibitor protein, IκB-α, which masks the nuclear localization sequences of p50/p65 heterodimer. IκB-α can be rapidly phosphorylated and degraded after stimulation with LPS or cytokines, allowing transcriptionally active p50/p65 heterodimers to translocate to the nucleus to activate a set of genes related to inflammation and proliferation. An alternative pathway to regulate p65 (Rel-A) is derived from its association with the unprocessed p105, the C-terminal portion of which bears a striking resemblance to IκB-α to form an inactive p105/p65 cytoplasmic complex. Processing of p105 results in a rapid degradation of the IκB-α homologous sequence and formation of transcriptionally active p50/p65 heterodimer. In both pathways, phosphorylation of IκB-α and p105 by a protein kinase(s) is essential for the subsequent degradation of IκB-α and the processing of p105, respectively. Resynthesis of IκB-α after its rapid degradation depends on the induction of IκB-α expression after NF-κB activation.\(^22\)

Cellular responses to cytokine signaling, such as activation of several protein kinases, including ceramide-activated protein kinase, p42/p44 mitogen-activated protein kinase, p38 mitogen-activated protein kinase, and Jun N-terminal kinase, have been suggested to exert their diverse biological consequences.\(^23\) However, little information is yet available as to how immediate signals by cytokines cause NF-κB activation in VSMCs. The present experiments using cultured rat VSMCs clearly demonstrated that stimulation with IL-1β caused a rapid phosphorylation of Ser^{32} of IκB-α and subsequent transient degradation of IκB-α, followed by NF-κB activation and its nuclear translocation. A cytokine-responsive IκB-α kinase recently identified phosphorlates two serine residues (Ser^{32} and Ser^{36}) of IκB-α.\(^24\) Phosphorylation of these residues is a prerequisite for polyubiquitination and subsequent degradation of IκB-α by 20S proteasome.\(^25\) TNF-α rapidly activates IκB-α kinase, peaking at 5 to 10 minutes in HeLa cells.\(^24\) In the present study, IL-1β induced a rapid (3 to 5 minutes) phosphorylation of Ser^{32} residue of IκB-α, followed by a transient (15 minutes) degradation and subsequent resynthesis of IκB-α in rat VSMCs. In the present study, a proteasome inhibitor, MG115, completely prevented the IL-1β-induced transient degradation of IκB-α and also stabilized the phosphorylated IκB-α induced by IL-1β in rat VSMCs. These data suggest that IL-1β rapidly stimulates IκB-α kinase to phosphorylate Ser^{32} and possibly Ser^{36} residues of IκB-α, which in turn is degraded via the ubiquitin/proteasome pathway, thereby leading to NF-κB activation in rat VSMCs. In fact, we have shown that the IL-1β-induced NF-κB activation and its nuclear translocation in rat VSMCs were completely prevented by pretreatment with MG115 (K.K. et al, unpublished data, 1998).

Our present study has clearly shown that NO donors (NOR3, SNP) inhibited the IL-1β-induced NF-κB activation and its nuclear translocation, accompanied by inhibition of both iNOS mRNA and protein expression. The present study has further shown that NO donor blocked both the transient decrease in IκB-α levels as well as its rapid phosphorylation induced by IL-1β, whereas NO donor added alone had no effect on basal IκB-α levels. Furthermore, NO donor abolished the appearance of IL-1β-induced phosphorylated IκB-α, even in the presence of a proteasome inhibitor, which stabilized IκB-α. These results suggest that the inhibitory effect of NO on the IL-1β-induced iNOS expression is mediated via inhibition of NF-κB activation primarily due to its inhibition of IκB-α phosphorylation rather than the proteasome-mediated IκB-α degradation. Our data are in agreement with those of previous reports showing the inhibitory effects of NO on TNF-α-induced NF-κB activation in endothelial and neuronal cells.\(^26,27\) However, our results appear to be in contrast to 2 recent (though contradictory) reports from the same laboratory.\(^17,28\) Liao and his associates have shown that NO donors inhibited TNF-α-induced NF-κB activation by induction and stabilization of IκB-α in human endothelial cells; S-nitrosothioglutathione (GSNO) prevented IκB-α degradation 30 minutes after stimulation with TNF-α.\(^22\) The same group has subsequently reported that GSNO did not prevent phosphorylation and degradation of IκB-α 15 minutes after stimulation with TNF-α in the same cells, suggesting that NO inhibits NF-κB activation by the late (2 hours) induction and nuclear translocation of IκB-α.\(^28\) In the present study, however, NOR3 itself had no effect on basal IκB-α expression during a 75-minute incubation period. The exact reasons for the apparent discrepancy between their reports and ours are unknown. This may be accounted for by the different cell types, species, cytokines, and NO donors used in the experiments. Among these, caution must be paid to the physicochemical nature of NO donors used, because they release NO with different kinetics and generate various metabolites that may affect cell functions. NOR3, (±)-(E)-ethyl-2-[(E)-hydroxyamino]-5-nitro-3-hexeneamide (FK409), is a novel and potent NO donor that spontaneously releases NO under neutral aqueous conditions with a half-life of 46 minutes.\(^29\) Although the possible involvement of the metabolite cannot be excluded, concentration-dependent inhibition of NF-κB activation by NOR3, with its greater
potency compared with other NO donors (such as SNP and GSNO) as demonstrated in this study, strongly suggests that the effect of NOR3 is due to the biologically active NO molecule.

The exact intracellular signaling mechanism by which NO blocks cytokine-induced NF-κB activation remains unknown. To address the question of whether the effect of NO is mediated by guanylate cyclase activation and subsequent cGMP generation, a cell-permeable cGMP analogue (8-bromo-cGMP) and a soluble guanylate cyclase inhibitor (ODQ) were tested. 8-Bromo-cGMP did not inhibit IL-1β-induced NF-κB activation or iNOS mRNA expression, whereas ODQ did not affect the NO-induced inhibition of either NF-κB activation or iNOS mRNA expression. These results argue against an intermediate role of cGMP and the involvement of cGMP-dependent protein kinase in the mechanism of NO inhibition of both NF-κB activation and iNOS expression.

NO modifies activities of several heme- and nonheme-containing enzymes by direct nitrosylation. For example, NO interacts with the heme moiety of soluble guanylate cyclase to stimulate its enzyme activity, whereas NO inhibits enzyme activity of heme-containing iNOS.30 NO also binds to nonheme-containing enzymes, particularly to iron-sulfur clusters (cis-aconitate, mitochondrial complexes I and II, ribonucleotide reductase), to inhibit their enzymatic activities.31–33 NO can also modify proteins by nitrosylation of Cys residue to form S-nitrosothiols.34 Therefore, it is possible to speculate that the inhibitory effect of NO on cytokine-induced NF-κB activation may be due to its direct nitrosylation of Cys residue(s) of IκB-α kinase to decrease its enzymatic activity. It is also possible that NO may inhibit NF-κB by dephosphorylation of IκB-α, because NO has been shown to activate protein phosphatases in monocytes.35 In fact, okadaic acid, an inhibitor of protein phosphatase 2A, has been shown to activate NF-κB and induce IκB-α phosphorylation.36 NO may function as an antioxidant to scavenge pro-oxidants, such as superoxide anion and hydrogen peroxide, which in turn stimulate redox-sensitive protein kinase(s) to activate NF-κB.37 However, antioxidants, such as N-acetyl-L-cysteine and pyrrolidinecarboxylate, failed to block IL-1β-induced NF-κB activation and IκB-α degradation in our cell culture (K.K. et al, unpublished data, 1998).

The NO-induced suppression of cytokine-induced IκB-α phosphorylation and degradation in rat VSMCs as demonstrated in the present study, along with the direct inhibition by NO on the enzyme activity of iNOS as recently reported,38 may constitute an auto-inhibitory mechanism to lessen the magnitude of NO-induced deleterious effects by high-output iNOS. Excessive NO production by augmented iNOS expression in the blood vessel, such as in inflammation, atherosclerosis, and septic shock, could be terminated not only via inhibition of iNOS enzyme activity, but also via transcriptional inhibition of iNOS gene by NO per se. Because NO inhibits cytokine-induced endothelial expression of adhesion molecules (vascular cell adhesion molecule-1, E-selectin, intercellular adhesion molecule-1) and proinflammatory cytokines (IL-1β, IL-8) via inhibition of NF-κB,39 the ability of NO to inhibit NF-κB to decrease iNOS gene expression in VSMCs may also contribute to the prevention of atherogenesis and inflammation in the vessel walls.

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References


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