Reciprocal regulation of the nitric oxide and cyclooxygenase pathway in pathophysiology: relevance and clinical implications

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Salvemini D, Kim SF, Mollace V. Reciprocal regulation of the nitric oxide and cyclooxygenase pathway in pathophysiology: relevance and clinical implications. Am J Physiol Regul Integr Comp Physiol 304: R473–R487, 2013. First published February 6, 2013; doi:10.1152/ajpregu.00355.2012.—The nitric oxide (NO) and cyclooxygenase (COX) pathways share a number of similarities. Nitric oxide is the mediator generated from the NO synthase (NOS) pathway, and COX converts arachidonic acid to prostaglandins, prostacyclin, and thromboxane A2. Two major forms of NOS and COX have been identified to date. The constitutive isoforms critically regulate several physiological states. The inducible isoforms are overexpressed during inflammation in a variety of cells, producing large amounts of NO and prostaglandins, which may underlie pathological processes. The cross-talk between the COX and NOS pathways was initially reported by Salvemini and colleagues in 1993, when they demonstrated in a series of in vitro and in vivo studies that NO activates the COX enzymes to produce increased amounts of prostaglandins. Those studies led to the concept that COX enzymes represent important endogenous “receptor” targets for amplifying or modulating the multifaceted roles of NO in physiology and pathology. Since then, numerous studies have furthered our mechanistic understanding of these interactions in pathophysiological settings and delineated potential clinical outcomes. In addition, emerging evidence suggests that the canonical nitroxidative species (NO, superoxide, and/or peroxynitrite) modulate biosynthesis of prostaglandins through non-COX-related pathways. This article provides a comprehensive state-of-the art overview in this area.

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at many levels, its central feature being modulation of molecular mechanisms that regulate PG-generating pathway. The final effect of this modulatory activity is complex. Endogenous NO, as well as NO-donors, stimulate or suppress the COX pathway depending on basal NO released, the cell type in which PG biosynthesis occurs, and the intensity of the stimulus employed for PG release. In addition, NO conjugates with superoxide anion (O$_2^-$, SO) to produce a potent proinflammatory and cytotoxic agent, peroxynitrite (ONOO$^-$), which also modulates COX enzymes. Taken together, these findings support an active reciprocal modulation of NO and PG-generating systems as a clinical rationale for using traditional NO donors and for developing novel therapeutic agents that target nitrooxidative stress (herein defined as stress caused by NO, SO, and/or PN) and the prostaglandin pathway.

Nitric Oxide Synthase and Cyclooxygenase Enzymes

Nitric oxide is generated via the oxidation of the terminal guanidino nitrogen of L-arginine by NOS (96, 97) (Fig. 1). In biological systems, NO is used as a generic term to encompass three redox-related species of NO include NO$^-$, which can modulate ferrous iron (Fe$^{2+}$) containing proteins by direct coordination to their Fe$^{2+}$ centers; such NO can be cytotoxic by reacting with superoxide anion to produce peroxynitrite. Further oxidation of NO species generates nitrogen dioxide (NO$_2$), dinitrogen trioxide (N$_2$O$_3$), dinitrogen tetroxide (N$_2$O$_4$), nitrite (NO$_2^-$), nitrate (NO$_3^-$), and others (152). The second important redox species of NO, nitrosonium ion (NO$^+$), can nitrosylate the thiol groups of various proteins, a modification that may serve important regulatory functions during pathophysiological states (35). Nitrosonium ion has an extremely short half-life (~$10^{-10}$ s) in solution at physiological pH and binds extremely rapidly to thiol groups. Following such binding, the resulting –SNO adducts maintain reactive “nitrosonium” characteristics and can transfer NO$^+$ to other thiols in a process called transnitrosylation (62, 72, 148). The third important redox-related species of NO, nitronyl (NO$^-$) anion, was reported to have a similar reactivity to NO$^+$ leading to S-nitrosylation (59); however, the normal or pathophysiological relevance of this species is unclear.

Three major isoforms of NOS have been identified on the basis of their relatively distinct primary amino acid sequences (only 50–60% identity), tissue and cellular distribution, and mode of regulation (96, 97). Two NOS isoforms are calcium/calmodulin dependent and are constitutively expressed (cNOS): endothelial-derived NOS, and neuronal derived NOS (nNOS) (96, 97). The third NOS isoform is cytokine-inducible and calcium/calmodulin-independent (iNOS) (96, 97). The distinct properties of each NOS isoform have important implications since it is the magnitude, duration, and the cellular sites of NO production that determines its overall physiological or pathophysiological effect. For example, release of NO from cNOS is localized and acts transiently. Nitric oxide released under these circumstances plays a crucial role in the cardiovascular and renal system, where it controls organ blood flow distribution, inhibits the aggregation and adhesion of platelets to the vascular wall, limits leukocyte adhesion and smooth muscle cell proliferation, promotes diuresis and natriuresis with the kidney (96, 97) and is involved in neurotransmission (10, 103). Most of these actions are mediated through the binding of NO to Fe$^{2+}$ in the heme prosthetic group of soluble guanylate cyclase, which catalyzes the conversion of GTP to cGMP (96, 97). In contrast, iNOS is expressed in a wide variety of host defense and other cells in response to inflammatory stimuli, such as endogenous cytokines and bacterial lipopolysaccharide endotoxin (LPS), resulting in a delayed (hours) but prolonged synthesis of high levels of NO. It is now well accepted that while NO released from iNOS is biologically appropriate, it is simultaneously involved in many pathological events (96, 97) (Fig. 1). The importance of Fe$^{2+}$ in mediating the functions of NO is also apparent when examining its cytotoxic effects, which are commonly observed when NO is produced in much larger quantities by stimulated macrophages, hepatocytes, and other cells. NO produced via such high-output systems inhibits proliferation of intracellular pathogens and tumor cells. These effects can be explained by the reactivity of NO$^-$ with Fe$^{2+}$ in the [Fe-S] centers of several important macromolecules, including aconitase and complex I and complex II of the electron transport chain (32, 50). The high affinity of NO$^-$ for Fe$^{2+}$ probably results in both the removal of Fe$^{2+}$ from [Fe-S] centers and the formation of nitrosyl Fe$^{2+}$ species within [Fe-S] proteins. Thus, NO exerts its function through its interaction with the Fe$^{2+}$ of heme and nonheme-iron-centered proteins.

Although NO$^+$ can interact with thiol groups of proteins to form S-nitrosothiols (72) in a nonenzymatically catalyzed S-nitrosylation reaction, there appears to be some specificity in this reaction. First, not all proteins containing free cysteines are likely to be S-nitrosylated, and not every available cysteine in a target protein is S-nitrosylated. For example, the ryanodine receptor contains 87 cysteines, but only 13 are normally targeted to be S-nitrosylated (178). Second, it has been postu-
lated that there is a primary consensus sequence for S-nitrosylation that requires acidic or basic residues adjacent to target cysteines (149). However, this presumptive acid-base consensus motif can also arise as a result of a target protein’s three-dimensional structure, as shown by analysis of the crystal structure of caspase-3, whose active cysteine is S-nitrosylated by NO in vivo (51). More than a hundred proteins have been identified as targets of S-nitrosylation associated with modification of their functions or structures including COX (35, 148).

Virtually all cells except erythrocytes can produce PGs from arachidonic acid that occurs at high concentrations among phospholipids of the cell membrane (Fig. 1). Prostaglandins are members of a lipid family that are enzymatically derived from polyunsaturated fatty acids with 20-carbon chains and four cis-double bonds (20:4) via the COX pathway. Prostaglandins are also known as “prostanoids”, which include classical prostaglandin, as well as the more specialized moieties thromboxane and prostacyclin (87, 144). The existence of biologically active PGs was first described in 1930 when Kurzrok and Lieb showed that fresh semen led to rhythmic contraction of human myometrium in vitro (65). Shortly thereafter, other groups independently confirmed the presence of an active substance derived from prostate glands that caused smooth muscle contraction and named it “prostaglandin” (41, 87, 169). Continued research has revealed that prostaglandins are a mixture of biologically active substances such as PGE or PGF, but ironically, these originate in the seminal vesicle and not the prostate gland (7).

One of the most important discoveries in the PG field occurred when Vane showed that the major anti-inflammatory effects of aspirin and other nonsteroidal anti-inflammatory drugs (NSAIDs) was due to their transient or irreversible inhibition of cyclooxygenase (165). Prostaglandins are formed by the action of prostaglandin synthase in a two-step conversion of arachidonic acid. First, the COX enzyme converts arachidonic acid to a cyclic endoperoxide (PGG_2), followed by peroxygenase cleavage of the PGG_2 peroxide to yield the endoperoxide (PGH_2) (105). These unstable intermediate products of arachidonic acid generated by COX are rapidly converted to other prostaglandins (PGE_2, PGF_2, TXA_2, PGI_2) by specific isomerases (Fig. 2). (105). COX was first purified as a 140-kDa homodimer from sheep seminal vesicles, which proved to be a structural homodimer, and yet exhibit functional heterogeneity that requires acidic or basic residues adjacent to target cysteines (149). However, this presumptive acid-base consensus motif can also arise as a result of a target protein’s three-dimensional structure, as shown by analysis of the crystal structure of caspase-3, whose active cysteine is S-nitrosylated by NO in vivo (51). More than a hundred proteins have been identified as targets of S-nitrosylation associated with modification of their functions or structures including COX (35, 148).

![Fig. 2. The scheme of COXs enzyme reaction mechanism. COX enzymes produce PGH_2 by two-step chemical reactions: cyclooxygenase and peroxygenase. PGH_2 is further converted to other prostaglandin by tissue-specific enzymes. Tyr, tyrosine; AA, amino acid; AH_2, nonspecific oxidizing agent that can donate 2 hydrogens.](http://ajpregu.physiology.org/)

Despite the structural similarity of the COX isoforms, their expression patterns and localization are very distinct. Constitutively expressed COX-1 is present in tissues such as the stomach, gut, or kidney, where PGs play a cytoprotective role in maintaining normal physiological processes. By contrast, COX-2 is expressed in many stimulated host defense cells at the site of inflammation, leading to robust production of proinflammatory PGs (142) (Fig. 1). Consistent with COX-2’s induction during inflammation, selective inhibition of COX-2 is both anti-inflammatory and anti-nociceptive (142). In addition, several groups have shown that upregulated COX-2 expression is involved in brain ischemia and that COX-2 inhibitors are neuroprotective (142). Such evidence led to the suggestion that COX-1 is homeostatically protective, whereas COX-2 may not be. However, this dichotomy is probably simplistic. For example, deletion of COX-1 in mice did not cause an increased susceptibility to gastric ulcer (67), while COX-2 inhibitors have been shown to attenuate the healing process (173). COX-1 is also expressed in quiescent inflammatory cells (18), whereas COX-2 is constitutively expressed in certain tissues, including brain and kidney, where it exerts protective effects (47, 67, 71, 180). Induced COX-2 has also been observed in endothelial cells; its inhibition with COX-2 inhibitors that spare COX-1 function in platelets has been held responsible for certain cardiovascular side effects observed with the usage of COX-2-specific inhibitors under chronic inflammatory conditions (100, 145). Moreover, utilizing a variety of COX inhibitors and sophisticated structure-functional analyses, it has been shown that COX enzymes form structural homodimers, and yet exhibit functional het-
erodimers, as inhibition of only one catalytic site is sufficient to block homodimer enzymatic activity, and binding of various fatty acids in a catalytic site can alter their enzymatic activities (118, 141, 168, 182–184). Furthermore, simultaneous expression of two isoforms of COXs led to an interesting observation by Yu et al. (182), demonstrating that COX-1 and COX-2 can form a heterodimer, which appears to play a critical role in the transformation that occurs in the blood vessels of newly born mice, implicating more complex roles and regulation of COX enzymes in biology.

Therefore, the NOS and COX systems are often coexpressed, share a number of similarities, and play fundamental roles in similar pathophysiological conditions (Fig. 3) (177). Increasing evidence suggests that there is considerable “cross-talk” between COX and NOS and that modulation of each respective pathway impacts the outcome of a physiological or pathological response. The interaction between NO- and PG-generating machinery occurs at multiple levels (92). Indeed, NO may interfere directly with COX expression and PG biosynthesis. On the other hand, AA and its metabolites generated by COX isoforms may also interfere with NO biosynthesis. Furthermore, expression of NOS seems to correlate with PG receptors and intracellular messengers, such as cAMP, generated by activation of GPCRs (92). Moreover, both NO and PGs interact with their own respective biosynthetic pathway by modulating molecular events underlying NOS and COX expression. Finally, some NO donors combined with several NSAIDs (nitroaspirin and nitroflurbiprofen, for example) have recently been synthesized and used in the treatment of many inflammatory and noninflammatory disorders, suggesting the possible beneficial effect of drugs acting on NO and PGs simultaneously (92).

What Are the Molecular Mechanisms Involved in the Regulation of Cyclooxygenase Pathway by NO and Related Nitroxidative Species

In 1993, Salvemini et al. (128) reported that NO increased the production of PGs at least in part by activating COX-1 and COX-2, findings that were subsequently corroborated by many groups in various in vitro and in vivo models (92). Using microsomal sheep vesicles (a rich source of COX-1), they demonstrated that exogenous administration of NO augments COX-1 activity, as well as murine recombinant COX-1 enzyme, leading to exaggerated increase in PGE$_2$ formation (Fig. 4) (127, 128). In addition to COX-1, NO activates COX-2. Indeed, using IL-1$\beta$-stimulated fibroblasts as a cellular model to explore the effects of exogenous NO on COX-2 activity, Salvemini and colleagues (127, 128) showed that exposure of IL-1$\beta$-stimulated fibroblasts to either NO gas or NO donors, such as sodium nitroprusside and glyceryl trinitrate, increased COX-2 activity several-fold and increased production of PGE$_2$ (Fig. 5) (127, 128). The effects of NO were cGMP-independent since methylene blue, an inhibitor of the soluble guanylyl cyclase (97), did not block the effects of NO (127, 128). These findings were soon confirmed by others (see, for example, Refs. 22, 53, 89, 91, 170, 174). The ability of NO...
Finally, the release of PGE2 by astroglial cells can be modulated by endogenously produced NO. Murine RAW-264.7 macrophages were stimulated with LPS to induce iNOS and COX-2 enzymes and produce large amounts of NO and PGs. As expected, inhibition of iNOS activity by nonselective and selective NOS inhibitors attenuated the release of NO from these cells (127, 128). However, the critical observation was that when NO release was inhibited, there was a simultaneous inhibition of PG release (127, 128). These results suggested that endogenously released NO from macrophages exerted a stimulatory action on COX-2 to enhance the production of PGs independently of the known effects of NO on the soluble guanylyl cyclase. As an extension, release of PGE2 by astroglial cells pretreated with N-methyl-D-aspartate (NMDA) is driven by activation of the l-arginine-NO pathway, an effect potentially relevant in pathophysiological mechanisms in which glutamatergic neurotransmission is involved (91). In addition, spontaneous release of PGE2 by hypoxic astrocytes occurs mainly via IL-1β-dependent activation of iNOS (93). Finally, the release of PGE2 by astroglial cells pretreated with IL-1β and TNF-α is due to enhanced COX-2 expression via activation of iNOS since release was blocked in the presence of a NOS inhibitor. These results may be relevant toward gaining a better understanding of the mechanisms underlying neuroimmune disorders.

When Salvemini et al. (128) proposed that NO increased PG formation by activating the COX enzymes, the group did not have a mechanistic explanation for how this occurred. So what have we learnt since 1993? It is unlikely that NO activates COX by binding directly to its heme prosthetic group, and several mechanisms have been proposed and depicted in Fig. 6. A first possibility is that NO acts as an antioxidant. Indeed, COX activity also provides a source of superoxide anion, and it has been postulated that superoxide could be involved in the autoinactivation of COX enzymes (33). NO interacts with superoxide and thereby limits the amounts of the superoxide radical that are normally necessary for auto-inactivation (43). Therefore, NO may augment COX activity by removing superoxide and acting as an antioxidant to prevent the autoinactivation of COX. A second possibility is NO-mediated formation of nitrosothiols, based upon the proposal by Hajjar et al. (46) that S-nitrosothiol may lead to NO-mediated activation of COX as it occurs in an heme-independent manner. On the other hand, Marnett et al. (78) investigated the role of free cysteines in COX-1 and showed that Cys313 and Cys540 mutations with serines alone inhibited its enzyme activity implying that NO-mediated Cys modification may interfere with COX-1 activity. Interestingly, both groups independently reported that SNAP, a NO chemical donor, could not directly activate COX-1 (66, 162). Thus confusion exists over whether S-nitrosylation of COX-1 leads to its activation. A third possibility is NO-induced generation of PN, the spontaneously generated product of SO and NO (5). Using purified COX-1 and COX-2 enzymes in vitro as well as excised sheep seminal vesicles, Landino et al. (66) reported that PN increased COX-1 and COX-2 activity. PN also stimulated COX-1 activity in aortic smooth muscle cells (161, 162). The mechanisms by which PN can activate COX enzymes remain undefined, but they could involve oxidative inactivation (78) or modification of key amino acids residues in the polypeptide backbone (2). Peroxynitrite can also oxidatively modify the COX substrate arachidonic acid, yielding F2-isoprostanes (76, 98). Peroxynitrite could block the activity of prostacyclin synthase and thereby attenuate the production of prostacyclin (189). A fourth possibility first proposed by Snyder and colleagues (58) is that iNOS itself binds to COX-2 and causes its S-nitrosylation at residue Cys-526, thereby enhancing its catalytic activity. This interaction and modification are specific for COX-2 as iNOS does not interact with COX-1. In particular, Kim et al. (58) observed more than one S-nitrosylated cysteine residue in LPS/IFN-γ-activated RAW264.7 cells and subsequently mutated each cysteine to a serine. In this way, they identified Cys-523, located near the catalytic domain, as the only cysteine that does not affect constitutive enzyme activity; when Cys-523 was mutated, NO no longer activated COX-2. Since Cys-523 is located near the catalytic site of COX-2, we speculated that this mutation leads to a slight conformational change in COX-2 that affects its enzyme kinetics (58). Indeed, Kim et al. (58) subsequently performed viscosity studies examining enzyme activity in various concentrations of sucrose and showed that release of product is accelerated in the presence of NO donor. However, it is not possible to exclude a potential involvement of other cysteines for NO-mediated activation of COX-2 as mutations of other cysteines alone affected the basal level enzyme activity of COX-2. Given that NO has a high affinity for heme, it is unclear how NO specifically S-nitrosylates the cysteines of COX-2 rather than interacting with its heme moiety. This may be partially explained by the fact that S-nitrosylation of COX-2 by iNOS requires physical interaction between the two proteins, specifically between the subunit-binding site of iNOS and the catalytic domain of COX-2 (Fig. 7A). Hence, Kim and coworkers speculated that NO generated from iNOS is simply in direct proximity of the S-nitrosylation target residue in COX-2 for immediate delivery and modification. Indeed, disruption of iNOS/COX-2 binding diminishes S-nitrosylation and activa-

![Fig. 6. Reciprocal regulation of PG biosynthesis by nitroxidative stress. NO can directly modify S-nitrosylate cysteine(s) in COX by direct protein-protein interaction between NOS and COX and activate it. COX also produces superoxide, which is speculated to be responsible for the autoinactivation of the COX enzymes. Rapid interaction between superoxide and NO reduces the levels of superoxide and produces PN, which can target either heme or tyrosine residues in COX. PN may interact with Fe in the heme group of COX and forms a radical intermediate product, which can accelerate the enzyme reaction while PN-mediated modification of Tyr-385 in COX can inactivate the activity of the enzyme. In those conditions, in which both the NOS and COX systems coexist and are coactivated, there can be a nitroxidative-mediated modulation in the production of PG resulting in beneficial or detrimental effects, such as increased inflammation and tissue injury.](http://ajpregu.physiology.org/doi/abs/10.1152/ajpregu.00355.2012)
tion of COX-2 in LPS-induced RAW264.7 cells (Fig. 7B). These studies elegantly demonstrated how NO generated from iNOS modulates COX-2 in macrophages but also raises additional questions. First, this interaction does not explain how exogenous administration of NO or NO donors increases PG generation in vitro. Indeed, a recent study by Tsikas and Niemann (159) showed that chemical donors of NO are unlikely to modify cysteines of recombinant COX-1/2 proteins in vitro, further corroborating importance of protein-protein interaction between iNOS/COX-2 for NO-mediated modification and activation of COX-2. Second, do both COX-2 in homodimer need to be S-nitrosylated to enhance its activity, as it has been shown that only one site of dimer is catalytically functional at any given time (183). We also do not know whether NO generated from iNOS can modulate COX-1 by either direct S-nitrosylation or transnitrosylation from COX-2 if a heterodimer is formed. Moreover, Goodwin et al. (42) demonstrated that removal of superoxide generated by LPS-activated RAW264.7 cells inhibited prostaglandin formation to the same extent as NO inhibitors. Whether these effects are mediated by SO or PN remains unknown. While the immediate speculation might be that PN-mediated nitrotyrosine formation of COX-2, Cross’s group showed that PN can also generate S-nitrosothiol (163). Therefore, further studies will be necessary to identify the form of NO-mediated modification of COX-2 in activated macrophages. S-nitrosylation of COX-2 does not seem to be limited to macrophages. In contrast to other tissues, COX-2 in the brain is constitutively expressed. We showed that COX-2 is S-nitrosylated in neuronal cells by nNOS (156). To our surprise, nNOS binding is mediated by its PDZ domain (postsynaptic density 95, PSD-95; discs large,Dlg; zona occludens-1, ZO-1; a common structural domain of 80–90 amino-acids found in the signaling proteins of various organisms), differing from the domain near the catalytic region that mediates iNOS binding to COX-2 (58, 156). We also showed that a physical interaction between nNOS and COX-2 is required for S-nitrosylation of COX-2 and that disruption of binding attenuated NMDA-mediated excitotoxicity, as did a selective COX-2 inhibitor (156).

In addition to effects on COX-2 enzyme activity, NO increases PG production from macrophages by acting posttranscriptionally or posttranslationally to increase their intracellular COX-2 protein levels (170). Studies using human osteoarticular chondrocytes revealed that NO released from sodium nitroprusside induced cell death, an event associated with DNA fragmentation, caspase-3 activation, down-regulation of Bcl-2, overexpression of COX-2, and increased release of PGE$_2$ (107). These events were abolished by blocking either the mitogen-activated protein (MAP) kinase pathway with the MAP kinase inhibitor PD98059, or the p38 kinase with inhibitor SB202190, as well as COX-2 activity, with the selective inhibitor NS-398. Although PGE$_2$ alone had no effect on cell death, it did sensitize chondrocytes to sodium nitroprusside-mediated death. These results suggest that NO activates both the extracellular signal-related protein kinase and/or the p38 kinase pathway, which, in turn, induce COX-2 and subsequent PGE$_2$ release. The latter may then sensitize human osteoarticular chondrocytes to an incipient NO-induced cell death (107). Similarly, interactions between NO and COX-2 and the signaling pathway involved were investigated in primary cultured rabbit cortical thick ascending limb cells (cTAL) (17). In these cells, immunoreactive COX-2 and vasodilatory prostaglandins were increased by exogenous application of the NO donor, S-nitroso-N-acetyl-l-$\text{d,L}$-penicillamine, and were associated with increased expression of phosphorylated p38. In addition, the administration of a low-salt diet to wild-type mice increased immunoreactive COX-2 and nNOS in the macula densa and surrounding cTAL of their kidneys, while the same diet did not significantly elevate COX-2 expression in nNOS$^{-/}$ mice. Collectively, the results demonstrate that in cTAL, NO can increase COX-2 expression in the macula densa through p38-dependent signaling pathways via activation of NF-$\kappa$B (17). NO is also necessary for maintaining prolonged COX-2 gene expression and sustained PGE$_2$ biosynthesis (117). NO-induced alterations in COX-2 gene expression were not related to changes in COX-2 mRNA (117, 155), and thus, the mechanism through which NO influences COX gene expression is not defined. Liu et al. (74) showed that NO can increase COX-2 mRNA levels via the $\beta$-catenin/TCF pathway leading to activation of the polyomavirus enhancer activator 3 (PEA3) transcription factor. Furthermore, NO also interacts with various other pathways that can influence COX expression, notably, the cAMP/PKA/CREB and JNK/Jun/ATF2 signaling cascades (74, 115). Recent evidence suggests that intracellular concentrations of NO and PGs may activate or suppress certain inflammatory cells by modulating endogenous biosynthesis...
and NOS/COX enzymes. Indeed, in human fetal microglial cells, very low concentrations of constitutive NO from cNOS are required to activate COX expression (55). Furthermore, low concentrations of NO enhance prostanoid formation, while higher concentrations downregulate the COX-2 pathway (151). Thus, endogenous or exogenous NO plays a crucial role in the regulation of COX activity, although the mechanism of this effect is still unclear. Evidence exists that in astroglia and other cells that possess both cNOS and iNOS, as well as in cells expressing only inducible iNOS, both NO and NO donors are able to regulate the expression of the constitutive and inducible COX enzymes (for review, see Refs. 20 and 21). In particular, NO released under basal conditions by cNOS maintains iNOS in a nonactivated state through the inhibition of the NF-κB signaling, which, in turn, regulates the iNOS transcriptional mechanisms. NF-κB represents one of the most relevant signaling pathways that mediate iNOS expression following many extracellular stimuli, including bacterial endotoxin and endogenous inflammatory cytokines (9, 11, 49, 80). When a low concentration of endogenous or exogenous NO is released, NF-κB remains inactive (157). Many factors may contribute in this NO-related inhibition of NF-κB, including the direct effect of NO on binding of NF-κB to its promoter response element, without affecting the activation and translocation of NF-κB (26, 83). Moreover, NO inhibits NF-κB binding to DNA through S-nitrosylation of the Cys-62 residue of the p50 subunit (26). Recent data show that NO interacts with NF-κB by stabilizing its endogenous inhibitor IkB-α. In particular, NO and NO− donors stabilize IkB-α by preventing its degradation from NF-κB and also increase the mRNA expression of IkB-α without affecting the mRNA expression of NF-κB subunits p65 or p50 (116). Recently, we have shown that agents such as tianeptine prevented an early downregulation of IkB-α (30 min) induced by HIV-1 coating gp120 in astrocytes needed for the activation of the classical NF-κB pathway and iNOS and COX2 induction (56). Thus, in cells whose iNOS is activated by extracellular inducers of NF-κB, NO may exert an early inhibitory role. Then, an increasing amount of NO combines with SO to generate PN, favoring derepression of NF-κB (possibly via nitration of IKK-β), resulting in the activation of iNOS. Since NF-κB is also a potent inducer of COX-2, and the action of NO on COX-2 is mediated by inhibition of NF-κB in mesangial cells (29), it is likely that these mechanisms may underlie some of the reported inhibitory effects of NO in prostaglandin biosynthesis.

Finally, COX enzymes are not the only target that can be modified by PN. Prostacyclin synthase is selectively inhibited by PN at low concentrations (IC50 = 50 nM), while thromboxane A2 synthase is activated at low concentrations too (187, 189). In particular, prostacyclin synthase contains one Cys at position 469, which is involved in heme binding, and a spectrophotometric study showed that this thiolate ligand at the fifth coordination position of the heme iron is not affected by PN. It appears that nitration of tyrosine residue 430 in prostacyclin synthase interferes with its activity (188, 189). iNOS-mediated regulation of prostaglandin production is not limited to COX enzymes. Xu et al. (179) showed that iNOS activates cytosolic phospholipase A2α (cPLA2α) by S-nitrosylation. In this situation, cPLA2α does not directly interact with iNOS, but rather forms ternary complex with COX-2 and iNOS. Hence, induction of COX-2 is a crucial step for S-nitrosylation and activation of cPLA2α (179).

In addition to this body of evidence showing the positive regulation of COX by NO, other reports support the idea that NO can inactivate COX under certain conditions. Indeed, Minghetti et al. (88) showed that LPS-induced COX-2 expression in microglial cells and their subsequent PG release are enhanced by co-incubation with NOS inhibitors. On the other hand, these results were confirmed by evidence that exogenous NO donors applied exogenously to LPS-treated microglial cells inhibit their PG release, an effect driven by reduced expression of COX-2 (44). This has also been found in other cell types, including vascular endothelial cells (30), rat Kupffer cells (147), and the J774 macrophage cell line (151). In addition, COX activation by inducible stimuli is driven in some models by mechanisms that do not seem to involve NO formation. This has been shown to occur, for instance, in astroglial cells treated with IFN-γ, where the elevation of PG levels and the expression of COX-2 have been found to not be affected by NOS activation (52).

At this stage, the relative roles of NO and PN on COX activation and/or induction remain to be defined, although there is no doubt that both species are involved at least in vitro. The relevance for PN as an activator/inducer of the COX enzymes in vivo has not been confirmed, mainly because selective PN decomposition catalysts (PNDCs) have not been available until now. Selective PNDCs have been designed and synthesized by Neumann and colleagues (31, 121, 122) for a recent series of elegant experiments. Studies are currently planned by our groups to use these probes as pharmacological tools to tease apart the roles of PN on PG biosynthesis. We have already demonstrated their use as potent anti-inflammatory agents, which are also able to block pain of various etiologies (31, 121, 122).

**NO Pathway Is Modulated by COX-Derived Products**

Products of the COX pathway may inhibit or increase the release of NO. High levels of PGI2 inhibit LPS-stimulated iNOS induction in J774.2 macrophages or murine peritoneal macrophages without affecting the enzyme once expressed (92). In rat Kupffer cells stimulated with endotoxin, inhibition of endogenously produced PG by indomethacin resulted in a concomitant inhibition of NO release (92) and conversely, indomethacin did not inhibit NO release in mouse RAW 264.7 cells or J774.2 macrophages (128). Thus, there are clearly cell-specific responses to PG exposure. The mechanism of action of PGs on the NOS pathway has been attributed in most instances to their activation of endogenous adenylate cyclases with subsequent increase in cyclic AMP (cAMP) levels. Increases in intracellular cAMP have been suggested as mediating either a stimulatory or an inhibitory role of PG on NOS pathway. For instance, in rat vascular smooth muscle, PKA activators such as forskolin or dibutylryl cAMP increases iNOS mRNA, as well as the formation of NO (92). Other studies have found an inhibitory influence of PGE2 on NOS expression. Thus, in cultured macula densa cells, PGE2 significantly reduced nNOS mRNA expression. In COX-2−/− mice, nNOS mRNA expression in kidney was upregulated throughout the postnatal periods. The induction of nNOS protein expression and NOS activity in COX-2−/− mice were localized to macula...
of NOS activity, it is not surprising that a reduction in intra-
platelet function, and inhibition of NOS and that Ca2+
inhibition of norepinephrine-mediated PGE2 release by the
by NOS inhibition and showed that this inhibition was due to
rulline and nitrite formation and indirectly by the measurement
of cGMP accumulation in platelets. The inhibitory effects of
aspirin and indomethacin on NO activity were reversed by
exogenous Ca2+, as well as by a functional TxA2 mimetic,
whereas a selective thromboxane A2 synthase inhibitor and the
 TxA2 endoperoxide receptor blocker exerted qualitatively sim-
ilar effects as COX inhibitors on NO activity. These effects were
not associated with a change in NOS protein expression
in platelets, suggesting that the effects of COX inhibitors are
mediated, at least in part, via TxA2 inhibition and Ca2+
mobilization in platelets (13). Because Ca2+ is a key regulator
of NOS activity, it is not surprising that a reduction in intra-
cellular Ca2+ by aspirin, indomethacin, or a selective TxA2
inhibitor results in decreased NOS activity (13). Thus, mobi-
lization of intracellular Ca2+ is a key step in the regulation
of platelet function, and inhibition of NOS and that Ca2+ mobil-
lization by COX inhibitors may serve as a regulatory step in the
interaction between the COX and NOS pathways.

When analyzed collectively, these diverse experimental data
on the inhibitory/stimulatory effects of PG on the NOS path-
ways are controversial and insufficient to draw any potential
conclusion on functional relevance.

Physiological Implications of the Modulation of the
Cyclooxygenase Pathway by Nitroxidative Species

The activation of COX-1 by intracellular or extracellular NO
released from eNOS has important consequences in normal
physiological conditions. Production of small amounts of NO
and PGs from the constitutive enzymes regulates various phys-
ological processes, including platelet aggregation, white blood
cell adhesion, blood vessel tone, cytoprotection in the kidney
and in the intestinal mucosa, renal nephron transport, tubulo-
glomerular feedback, and renin release. The main difference
between NO and PGs in mediating these effects lies at the level
of their respective intracellular transduction mechanisms.
Thus, NO activates soluble guanylate cyclases to increase
cGMP, while PGs activate adenylate cyclase to increase
cAMP. Another interesting effect of NO acting upon COX-1
may be the complex regulation of neuropeptide release. Nor-
epinephrine mediates the release of luteinizing hormone-re-
leasing hormone (LHRH) from its terminals, a process known
to require increased release of PGE2 (123). In an elegant study,
Rettori et al. (123) demonstrated that LHRH release from
norepinephrine-stimulated hypothalamic slices was prevented
by NOS inhibition and showed that this inhibition was due to
inhibition of norepinephrine-mediated PGE2 release by the
NOS inhibitors. These results raise the possibility that NO
through its ability to activate COX, may mediate exocytosis
of secretory granules for other neuropeptides that are known to be
regulated by PGE2. NO-driven COX-1 activation also affects
reproduction in that NO modulates uterine motility (36) by
activating COX-1 and releasing PGE2. Moreover, the recent
observation of the dual inhibition of NO and PG by nonselect-
ive NOS inhibitors may well explain the clinically deleterious
effects caused by these drugs in kidneys and the gastrointesti-
nal tract, where both NO and PG are considered to be cyto-
protective. As initially discussed by Wallace (173), there is
ample evidence that selective inhibitors of COX-2 produce less
gastric damage than standard NSAIDs when administered
acutely to healthy animals. However, most patients taking
standard NSAIDs do not develop clinically significant gastric
injury, although a subset of patients is more susceptible to
the gastric-damaging actions of these drugs (54). Moreover,
the presence of both NOS isoforms in the vasculature raises
the question of which is the predominant cause of the increased
production of vasodilatory PGs that are critical to preserving
renal blood flow during volume depletion and dehydration
stress. Inhibition of this homeostatic response accounts for the
most common renal side effects associated with nonselective
NSAID therapy (102). In particular, it has been demonstrated
that combinations of NSAIDs, taken in large doses over a
prolonged period, cause renal papillary necrosis and interstitial
scarring (63). Such patients develop chronic renal failure and
are susceptible to the subsequent development of uroepithelial
tumors. It is not clear what comprises the mechanism of
NSAID toxicity; theories include the effects of COX inhibition
and direct toxicity owing to elevated concentration of the drugs
in the medulla, anoxia, and metabolic effects (8). During
endotoxemia, PGE2 derived from COX-1 and COX-2 appar-
ently exerts an inhibitory effect on gastric iNOS, despite no
change in iNOS protein or mRNA. Thus, this PGE2 may
represent a cytoprotective mechanism for iNOS, since upregu-
lation of iNOS and generation of toxic byproducts, such as PN
is deleterious (160, 176). As an extension to these studies,
Franco and Talamini (37) reported that COX-2 inhibition in
human gastric biopsies during Helicobacter pylori infection
decreased the production of PGE2 and NO, but iNOS inhibitors
did not modulate PGE2 generation. It is, therefore, possible that
inhibition of NO production following COX inhibition contrib-
utes to gastrointestinal issues associated with the use of both
selective and nonselective COX inhibitors (171, 173).

The findings that NO activates COX-1 enzyme has uncover-
red additional mechanisms by which NO donors might exert
beneficial clinical effects. As one important example, in the
presence of an intact endothelium, antplatelet NO normally
keeps blood vessels dilated, and in conjunction with PGI2,
maintains the nonthrombogenic nature of the endothelium.
Disease states that damage the endothelium or alter the normal
properties of the endothelial cells lead to a decrease in the
production of NO, tipping the balance in favor of constriction,
thrombosis, and vasoocclusion.

Diseases associated with locally impaired release of NO
include coronary artery spasms that occur either spontaneously
(Prinzmetal angina) or in the context of atherosclerosis, myo-
cardial ischemia, and stroke (166). Theoretically this imbal-
ance can be restored by exogenous NO donors, which should
be uniquely beneficial in conditions with endothelial dysfunc-
tion because they preempt a failing endogenous system. Since
NO donors do not require an intact endothelium to be effective,
they will restore vascular dilation and suppress the tendency to platelet aggregation. Although the vascular effects of NO donors have been well documented, these compounds are also endowed with antiplatelet effects and antithrombotic properties. NO donors inhibit platelet adhesion and aggregation in vitro and in vivo (24, 95, 130), increase bleeding time in humans (70), and synergize with PGI2 (61, 95). Furthermore, NO donors potentiate the activity of thrombolytic factors in providing further protection against vascular occlusion (61). NO donors that release NO spontaneously are far more potent inhibitors of platelet function in vitro compared with those requiring bioconversion. Platelets do not have the enzyme to convert organic nitrates to NO, and therefore, in vitro, they will respond only to very high doses of nitrates (1). The fact that endothelial cells can metabolize organic nitrates, such as glycercyl trinitrate (GTN), as efficiently as smooth muscle cells (6, 34, 129), is important because it provides the key to understand how organic nitrates inhibit platelet function in vivo, whereas they generally display weak anti-platelet effects in vitro (6, 75, 129).

The findings that smooth muscle cells and endothelial cells significantly increase the in vitro antiplatelet effects of GTN (where the dose of GTN required to inhibit platelet aggregation in vitro approximates that used in vivo) led to the proposal that the increased effectiveness of organic nitrates in vivo as antiplatelet agents is due to conversion to NO by cells in the vascular tree, such as smooth muscle cells and endothelial cells (6, 34, 129). In this respect, endothelial cells may be more important because of their potentially direct contact with platelets. NO donors also promote the release of PGI2 in vivo and in vitro from endothelial cells (25, 69, 84, 138). The latter effect presumably reflects the ability of NO donors to activate the cyclooxygenase pathway directly, a phenomenon associated with a marked release of prostaglandins (128).

Pathological Implications of Modulating the Cyclooxygenase Pathway by Nitrooxidative Species

In vivo studies reveal that regulation of COX by NO is a powerful mechanism by which NO modulates the course of the inflammatory response. Thus, iNOS and COX-2 are induced in a number of inflammatory models, including rabbit hydrenephrotic kidney (131, 139), endotoxin-induced septic shock (132, 150), inflammation (45, 57, 86, 126, 134, 136, 137, 167, 186), ischemia-reperfusion injuries, and pain (82, 104, 134, 146). In these settings, inhibition of NO inhibits both NO and PG release, and the anti-inflammatory potency of the iNOS inhibitors correlates with their respective ability to block both NO and PGs. For instance, in the acute inflammation of carrageenan-induced paw edema in rats, inhibition of edema with N6-(1-iminoethyl)-L-lysine (L-NIL) correlates with dose-dependent inhibition of NO synthesis and formation of proinflammatory PGs (134). That the proinflammatory role of NO involves prostaglandins has also been demonstrated by injection of sodium nitroprusside to elicit edema in the footpad of rats, a response that is attenuated or blocked by NSAIDS (137). Therefore, the effects of endogenously released NO on COX-2 are mimicked by exogenous NO. In acute pancreatitis, the increased production of PGs was inhibited by NO synthase inhibitors, indicating the participation of NO in this process (19). Furthermore, injection of carrageenan into the preformed air pouch of a rat induces an inflammatory response characterized by iNOS and COX-2 induction, white blood cell infiltration, edema, and protein leakage into the pouch (126). Selective inhibition of iNOS (or other iNOS inhibitors) by L-NIL or aminoguanidine inhibited not only NO but also PG production. All other parameters of inflammation were attenuated, and histological examination of the pouch lining from animals that were dosed in vivo with the L-NIL revealed a lack of inflammation (126). Additional studies also reported a cross talk between iNOS and COX-2 in the lung of rats given endotoxin; release of PGI2 from lungs harvested from endotoxin-treated rats in the presence of an iNOS inhibitor was significantly attenuated compared with control animals (136). Importantly, targeted deletion of iNOS in mice decreases COX-2 activity (79), clearly strengthening the tie between NO and PN and eicosanoid biosynthesis. Injection of NO donors, such as sodium nitroprusside or nitroglycerin, into the air pouch activates COX-2, resulting in profound increase in PGE2 release (126). Treatment of diabetic rats with nitro-L-arginine methyl ester (L-NAME) prevents the up-regulation of renal COX-2 and the associated increase in renal PG release in response to arachidonic acid, suggesting that NO or a product of NO may contribute to the induction of COX-2 in diabetes (14). Such induction of COX-2 in diabetes may contribute to renal functional and structural changes associated with this condition, as inhibition of COX-2 reduces GFR in the diabetic rat (60) and other markers of renal injury in hypertensive diabetic rats (12). In this content, it is well documented that COX-2 and nNOS are coexpressed in macula densa cells, and their coexpression is stimulated in a number of high-renin states. In particular, plasma renin activity was significantly reduced in nNOS−/− mice of an otherwise mixed genetic background and in COX-2−/− mice with either BALB/c or C57BL6 backgrounds (112). Additional findings further implicate important interactions between the NO and COX-2 systems in the regulation of arteriolar tone and the renin-angiotensin system by the macula densa (16, 48).

The interaction between iNOS-derived NO and COX-2 has raised the possibility that some deleterious effects of ischemia-induced iNOS expression are mediated through COX-2 reaction products, in brain and bladder, for example (81, 101, 106). The important interaction between iNOS-derived NO and COX-2 in cerebral ischemia was described for the first time by Iadecola and colleagues (106). Although iNOS-positive neutrophils were found in close spatial proximity to COX-2-positive neurons, inhibition of iNOS by aminoguanidine was found to attenuate COX-2 reaction products only in the ischemic region (106). Furthermore, the authors demonstrated that postischemic PGE2 production is reduced in nNOS−/− mice despite normal COX-2 mRNA and protein expression (106). These observations led them to propose that in the ischemic brain, NO produced by iNOS drives COX-2 activity and increases its catalytic output (106). In a subsequent study, Nagayam et al. (101) assessed the relative contribution of iNOS and COX-2 to ischemic brain damage. They reasoned that if the pathogenic effects of iNOS-derived NO and COX-2 are mediated through largely independent mechanisms, then inhibition of COX-2 in iNOS-null mice (iNOS−/−) should confer a protection greater than that attained by iNOS deletion alone. Conversely, if iNOS-derived NO mediates some of its deleterious effects through COX-2, then inhibiting COX-2 in iNOS−/− mice should not provide added protection. Using the selective COX-2 inhibitor, NS398, the authors reported that inhibition of COX-2 attenuates ischemic brain damage in
wild-type mice but not in iNOS−/− mice, thus supporting the hypothesis that COX-2 activation is an important pathogenic factor in the damage produced by iNOS-derived NO (101). In a model of bladder ischemia-reperfusion (I/R), NO derived from up-regulated iNOS after I/R increased COX-2-derived PG via a cGMP-independent mechanism. Thus, NO-mediated activation of COX-2 may be an important mechanism for the modulation of bladder function after I/R injury (81). Another interesting interaction between the NOS and COX pathways occurs in pain. For example, inhibiting the antihyperalgesic effects of NO synthases inhibitors in models of inflammation is associated with inhibition of both NO and PGs. NO synthases inhibitors (73) have also been shown to attenuate spinal release of excitatory amino acids, such as glutamate and PGE2, following NMDA receptor activation (146). In addition, Matsui et al. (82) demonstrated that activation of Toll-like receptor 4 in spinal cord microglial cells released both NO and PGE2 following activation of p38 kinase. This was attenuated by NO synthases inhibitors, while COX inhibitors had no effect on NO (82).

A specific area of interest in which the simultaneous modulation of NO and COX has been recently investigated is the development and prevention of neoplasias, notably colon cancer. Colorectal carcinoma remains a major cause of cancer patient mortality worldwide. The development, progression, and metastasis of this carcinoma follow a well-defined series of steps, apparently including a dependence on local NO concentration and overexpression of cyclooxygenase enzymes (111, 119, 120, 154). In particular, at low levels, NO increases tumor growth and development, while higher levels of NO (above optimal concentrations) may express cytotoxic or cyostatic effects (140). In addition, the degree of tumor malignancy is also linked to the amount and activity of NO synthases proteins (39), and the expression of NO synthases may promote metastatic behavior of tumor cells. Moreover, the level of nitrite and nitrate in serum or culture supernatants can be significantly reduced by application of L-NAME, a competitive inhibitor of NO synthases. Its activity is based on structural similarity to L-arginine, a substrate for NO synthases, and competitive inhibition of L-arginine metabolism. It has been shown that L-NAME exerts anti-invasive and antimetastatic effects on many cancers, including colon carcinoma (181). On the other hand, COX-2 has been suggested to play an important role in carcinogenesis and opens new anti-cancer therapeutic possibilities (40, 185). Furthermore, evidence exists that NS398 may in a dose-dependent manner inhibit proliferation and induce death in many carcinomas, and the expression of NOS may promote metastatic behavior of cancer cells, their reciprocal interactions, and the local conditions of the microenvironment. The knowledge of these effects may be useful in limiting progression and invasion of colon carcinoma.

**Perspectives and Significance**

Compelling data have been generated over the last 18 yr supporting the concept that NO is involved in the regulation of COX pathway and vice versa. In particular, basal release of both NO and PGs appears to be vital in the maintenance of a basal “protective” homeostasis in both vascular and nonvascular tissues against exogenous, as well as endogenous insults. This occurs via inducible NOS and COX enzymes that when activated during inflammatory states, release micromolar concentration of both mediators. On the other hand, disruption of NOS/COX cross-talk with the simultaneous release of large quantities of NO and PGs represents key events in tissue damage occurring in a diverse array of inflammatory and degenerative processes. Such a process may have significant implications for the regulation of key pathophysiological events, including blood flow and oxygen delivery, smooth muscle cell modulation in vascular and nonvascular tissues, immune response, and neuron/glia interaction. Furthermore, the finding that COX inhibition via NSAIDs has a significant effect on vascular tone during hypoxia only after prior inhibition of NOS with L-NAME, suggests a compensatory response and by retarding tumor growth and metastasis (109), producing beneficial effects in chemically induced colorectal carcinomas by influencing host immune system functions (77). Experimental supplementation with this amino acid also leads to increased NO formation by the oxidative deaminase pathway. In turn, an increased local NO level may induce cytostasis by inhibiting hyperproliferation of tumor cells or may be cytotoxic to tumor cells by the formation of toxic peroxynitrite (77). L-arginine was also recently found able to decrease PGE2 levels in cocultures of tumor spheroids with colonic epithelium. These results suggest an important role of NO in chemoprevention of tumor-epithelial cell interactions rather than its effect on tumor-stromal development, activity, and viability. In addition, NO was found to inhibit COX-2 enzymatic activity by reacting with iron in the enzyme’s heme group. Moreover, L-NAME, an inhibitor of NO synthases, which decreased the NO level and motility of tumor cells under in vitro experimental settings, has a relatively weak effect on COX-2 level. These apparently contradictory results reflect that any NO and COX-2 relationship depends upon cell types that have been used, their direct interactions, the kind of inhibitor applied, and the local microenvironmental conditions. These elements regulate NO effects on COX-2 and PGE2 synthesis in normal as well as in cancer cell lines. Indeed, it has recently been reported that mucosal PGE2 in noncancer cell lines is decreased by SC-560, a COX-1 selective inhibitor (143), but not by L-NAME (108). On the other hand, L-NAME reduces PGE2 generation by attenuating COX-2 expression (113), which may limit the invasion and migration of tumor cells (181). Thus, consistent cross-talk among NO, COX-2, and PGE2 exists in colorectal cancer cell lines. This relationship is rather unidirectional where NO level influences COX-2 and PGE2 levels. Moreover, any imbalances in NO level caused by exogenous factors influence COX-2 and PGE2 amounts depending on the kind of cells, their reciprocal interactions, and the local conditions of the microenvironment. The knowledge of these effects may be useful in limiting progression and invasion of colon carcinoma.
by PGs to maintain the vasodilator response to hypoxia, which is tightly NO dependent.

A similar conclusion can be drawn when evaluating the role of NO and PGs in neurodegenerative processes. In brain tissues, in which cellular populations coexist that express both constitutive and iNOS and COX enzymes (e.g., microglial cells and astrocytes), early stages of HIV-related brain damage are characterized by down-regulation of constitutive NOS and COX that, under basal conditions, tend to counteract the massive diffusion of HIV-related neurotoxins into brain tissue. Under these conditions, it is likely that the inhibition of both NOS and COX aggravates the neurodegenerative process and enhances neuronal cell death. Conversely, the overproduction of free o xo radicals, species with elevated levels of NO, leads to generation of PN that then modulates NF-κB via IKK-B, leading to “intensive” activation of iNOS and COX-2. This activation seems to be the key event in generating toxic concentration of both mediators, as shown by evidence that the combined effect of NO donors and NSAID, at this stage, exerts a beneficial effect on HIV-related cognitive dysfunction (56, 94). That this represents a more general reactive model for the cooperation of NOS and COX enzymes is clearly expressed by changes of protective enzymes in astroglial cells accompanying NOS/COX interaction. For example, glutamine synthase, the enzyme that depletes the synaptic space of secreted glutamate by converting this mediator into nontoxic glutamine, is regulated in the same way by NO and PGs. In particular, neurotoxic insults (e.g., HIV coating gp120 glycoprotein), which occur by modulating basal NO/PGs release, also decrease glutamine synthase expression via this mechanism, an effect restored by NO-NSAIDs. When large quantities of NO/PGs are generated via iNOS and COX-2, GS is unable to remove right quantities of glutamate from periastroglial space leading to apoptotic cell (56). Thus, maintaining adequate basal concentrations of NO/PGs mechanisms serves not only to keep iNOS and COX inhibited, but also to protect specific tissues against toxic insults, including oxidative stress. This is relevant not only for the understanding of pathophysiological events involving NO/PGs mechanisms but also for the choice of right treatment to be performed based on NO/PG modulation.

It remains to be better understood how the protective effect of NO/PGs basal synthesis mechanisms is disrupted under pathological conditions, which then lead to widespread activation of toxic mechanisms. In this respect, the formation of PN seems to represent a key event in the shift of NO/PGs mechanisms into toxic behavior.

Current data indicate that NO modulates eicosanoid production at several levels, and it is increasingly recognized that more than one enzyme in the arachidonic acid pathway can be affected by NO and/or PN. At this stage, it is difficult to determine whether effects of NO on PG production are mediated by NO itself or its byproduct, PN; currently available NOS inhibitors block both NO and PN formation (73, 152). Clearly, PN can activate COX-1 and COX-2 and can induce COX-2 (73, 152). In this regard, we recently reported that intraplantar administration of PN in rats causes peripheral sensitization and pain following activation of COX-1 and COX-2, as well as the NF-κB-induction of COX-2 leading to increased formation of PGE2. In this model we found that the decomposition of elevated PN levels using a PNDC, such as FeTMPyP [Fe(III)5,10,15,20-tetrakis(N-methylpyridinium-4-yl)porphin]] (125, 133), signifi-


CROSS-TALK BETWEEN THE NITRIC OXIDE AND PROSTAGLANDIN PATHWAY


