Balloon Injury and Interleukin-1β Induce Nitric Oxide Synthase Activity in Rat Carotid Arteries

Ghislaine A. Joly, Valérie B. Schini, and Paul M. Vanhoutte

Experiments were performed to investigate whether balloon injury induces nitric oxide synthase activity in the blood vessel wall. Contractions to phenylephrine were compared in left carotid arteries of the rat, previously injured by balloon catheterization and excised either immediately (t=0), 6, or 24 hours after the procedure, with those in control right carotid arteries (with and without endothelium). Phenylephrine evoked comparable concentration-dependent contractions in balloon-injured (t=0) and control carotid arteries without endothelium, whereas those in control arteries with endothelium were depressed. In the balloon-injured carotid arteries (6 and 24 hours), the concentration–contraction curves to phenylephrine were shifted to the right compared with those observed in balloon-injured arteries (t=0). In balloon-injured carotid arteries (6 hours), the hyporeactivity to phenylephrine was enhanced by superoxide dismutase. In balloon-injured carotid arteries (24 hours), nitro-L-arginine and methylene blue restored full contractions, whereas superoxide dismutase potentiated the hyporesponsiveness to phenylephrine. The depressed contractions were associated with a concomitant increase in the basal level of cGMP; this production was abolished by nitro-L-arginine. The depression of the concentration–contraction curves to phenylephrine and the increase of the tissue level of cGMP induced by interleukin-1β (4 hours) were more pronounced in balloon-injured arteries (24 hours) than in control arteries without endothelium. The effects of interleukin-1β were inhibited by nitro-L-arginine. These observations indicate that in vivo endothelial injury of the rat carotid arteries induces the production of nitric oxide from L-arginine in the blood vessel wall, an effect which is potentiated by interleukin-1β. (Circulation Research 1992;71:331–338)

Key Words • balloon injury • rat carotid artery • interleukin-1β • atherosclerosis • nitric oxide

Endothelial cells produce nitric oxide, which relaxes vascular smooth muscle1–3 and inhibits platelet adhesion and aggregation.4–6 The effects of nitric oxide on vascular tone and on platelet aggregation are mediated, in part, by cGMP.5,7–13 Nitric oxide is synthesized by oxidation of the terminal guanidino-nitrogen atom(s) of l-arginine by nitric oxide synthase(s).14,15 In endothelial cells, nitric oxide synthase activity is expressed constitutively, is associated with the cytosol and particulate fractions, and requires NADPH and Ca2+-calmodulin for nitric oxide synthesis.16–18 A second, inducible form of nitric oxide synthase has been identified in endothelial cells,19,20 macrophages,21 neutrophils,22,23 and vascular smooth muscle.24 This enzyme is induced by cytokines such as interleukin-1β (IL-1β)19,20,25,26 and endotoxins.27,28 The activation of the inducible enzyme subtype is Ca2+ independent and requires NADPH and tetrahydrobiopterin for nitric oxide synthesis.29

Angioplasty, which is a common procedure used to restore flow in stenosed arteries, induces endothelial injury.30,31 Early after this procedure, the surface of the arteries exhibits platelet thrombi, which adhere to the injured surface. However, several hours later, the injured arteries are covered only with a monolayer of platelets.32,33 The destruction of the endothelial cells is also associated with the recruitment and activation of other blood cells, such as neutrophils and monocytes,34 presumably because of their exposure to subendothelial connective tissue. The activation of blood cells induces release of important mediators of immune and inflammatory responses, such as IL-1β,35,36 growth factors,37 and oxygen-derived free radicals.38,39

The aim of the present study was to investigate whether endothelial injury by balloon catheterization is associated with an induction of nitric oxide synthase activity in the blood vessel wall.

Materials and Methods

Male Wistar rats (300–400 g) were anesthetized by intraperitoneal injection of ketamine (100 mg/kg). The carotid arteries were excised and stored in a cold modified Krebs-Ringer bicarbonate solution containing (mM) NaCl 118.3, KCl 4.7, MgSO4 1.2, KH2PO4 1.2, CaCl2 2.5, NaHCO3 25.0, CaEDTA 0.016, and glucose 11.1 (control solution). Two types of carotid arteries without endothelium were prepared: one group, re-

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Supported in part by National Institutes of Health grants HL-31183 (P.M.V.) and HL-46356 (V.B.S.) and by an unrestricted Research Award from the Bristol-Myers Squibb Research Institute.

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Received December 16, 1991; accepted March 26, 1992.
ferred to as “control artery without endothelium,” consisted of the right common carotid artery. In these preparations, the endothelium was removed mechanically by inserting a small metal wire into the lumen and rolling the tissue back and forth several times on a paper towel wetted with control solution. The second group, defined as “balloon-injured arteries,” consisted of the left common carotid artery. In this group the endothelium was injured in vivo in anesthetized rats (ketamine, 100 mg/kg i.p., and chlorpromazine, 3 mg/kg i.p.) by inserting a Fogarty embolectomy catheter (2F) into the external carotid artery; the catheter was then passed down the common carotid to the level of the aorta. The uninfated balloon catheter was moved back and forth seven times through the entire length of the carotid. The catheter was removed, and the external carotid was ligated. Blood flow was maintained via the internal carotid branch that was not subjected to any surgical procedure (following protocol no. 91473 approved by the Institutional Animal Care Committee of Baylor College of Medicine). The balloon-injured carotid arteries were collected immediately (t=0), 6, or 24 hours after the injury procedure, cleaned of connective tissue and fat, and suspended in organ chambers. In all experiments, the contralateral right carotid artery without endothelium was used as a control blood vessel. In some experiments vascular reactivity to phenylephrine and relaxing effects of 3-morpholinosydnonimine (SIN-1) (a donor of nitric oxide) were tested in the control right carotid artery with endothelium. The rings from the arteries were stretched progressively to 1 g of tension (optimal length for maximal contraction as determined in preliminary experiments with phenylephrine 10^{-6} M; data not shown) in organ chambers containing 20 ml of control solution (37°C, pH 7.4) and bubbled with 95% O_2–5% CO_2. Changes in isometric tension were recorded with an isometric force transducer (Celaester, Poitiers, France). The presence or absence of the endothelium was verified by addition of acetylcholine (10^{-6} M) and calcium ionophore A23187 (10^{-6} M) in arteries contracted with phenylephrine (10^{-6} M or 10^{-7} M, respectively) and by histology (see below). The carotid rings were rinsed three times with warm control solution, and after a resting period (30 minutes) they were incubated for 30 minutes either with solvent, nitro-L-arginine (NLA) (10^{-4} M; an inhibitor of nitric oxide synthesis^{40,41}), L-arginine (10^{-4} M), superoxide dismutase (SOD) (100 IU/ml; a scavenger of superoxide anions^{42,43}), or methylene blue (10^{-5} M; an inhibitor of soluble guanylate cyclase^{44,45}). Next, a concentration–relaxation curve to phenylephrine (10^{-9}–10^{-5} M) or a concentration–relaxation curve to SIN-1 (10^{-9}–10^{-5} M) in rings previously contracted with phenylephrine (10^{-5} M) was obtained. All experiments were performed in the presence of indomethacin (10^{-5} M) to prevent the synthesis of vasoactive prostanooids. To induce nitric oxide synthase activity, rings from control (without endothelium) and balloon-injured carotid arteries were incubated in culture medium (minimum essential medium containing 2 mM glutamine, 20 mM N-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid-NaOH, 20 mM N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid-NaOH [both pH 7.3], 100 IU/ml each streptomycin and penicillin, and bovine serum albumin 0.1%) in the presence of IL-1 β (30 IU/ml) for four hours at 37°C in a cell culture incubator before suspension in the organ chambers.\textsuperscript{10,26}

**Tissue Content of cGMP**

Rings were incubated in warm (37°C) control solution (5 ml) containing indomethacin (10^{-5} M) and 3-isobutyl-1-methylxanthine (IBMX; 10^{-4} M; a nonselective inhibitor of phosphodiesterases\textsuperscript{46}) for 30 minutes to inhibit the production of prostanooids and the degradation of cyclic nucleotides by phosphodiesterases, respectively. During this period, rings were incubated with NLA (10^{-4} M) or solvent. Nitric oxide synthase activity was induced in both types of preparations by incubating the rings in culture medium (for composition see above) containing IL-1 β (30 IU/ml) for 4 hours at 37°C in an incubator prior to their equilibration in control solution containing indomethacin and IBMX for 30 minutes. They were frozen quickly with an aluminum clamp cooled in liquid nitrogen. Subsequently, rings were homogenized in 1 ml of 6% trichloroacetic acid, sonicated for 5 seconds, and centrifuged for 15 minutes (13,600g). Supernatants were extracted with 4 vol water-saturated ethylether before being lyophilized. Each sample was resuspended in 0.3 ml of sodium acetate buffer (0.05 M, pH 6.2), and the content of cGMP was determined using a cGMP kit (Biomedical Technologies Inc., Stoughton, Mass.), with an acetylation step included to increase sensitivity. After lyophilization the pellets were incubated with NaOH (0.3 ml of 0.1N) at 37°C for 24 hours, and the protein concentration of each sample was determined using BCA Protein Assay Reagent (Pierce Chemical Co., Rockford, Ill.).

**Morphology**

Representative sections of the left and right carotid arteries were fixed in 10% neutral buffered formalin (Surgipath Medical, Grayslake, Ill.). Following fixation, the tissues were trimmed, dehydrated in an ascending series of graded ethanol, cleared in xylene, and infiltrated with paraffin wax (Paraplast X-TRA, Sherwood Medical, St. Louis, Mo.). Processing of tissue was performed on a Miles Laboratory VIP 3000 tissue processor. Paraffin blocks were sectioned at 3–5 μm on a standard rotary microtome (Leitz 1512), and the sections were recovered from a water bath on acid-alcohol–cleaned slides. Sections were stained with hematoxylin and eosin using an automated staining system (Fisher Scientific CODEON System). Cross sections were examined by light microscopy (Olympus, AH.3).

**Drugs**

Phenylephrine hydrochloride, acetylcholine chloride, calcium ionophore, indomethacin, IBMX, methylene blue, SOD, and L-arginine were purchased from Sigma Chemical Co., St. Louis, Mo. Human recombinant IL-1 β was obtained from Boehringer Mannheim, Indianapolis, Ind., and NLA from Aldrich Chemical Co., Milwaukee, Wis. SIN-1 was a gift from Laboratoires Hoechst, Paris. Indomethacin was prepared in an equimolar concentration of sodium carbonate (10^{-5} M). All other drugs were prepared in distilled water.

**Statistical Analysis**

Results are expressed as mean±SEM. The number of rats studied is represented by n. The negative logarithm
of the effective molar concentration causing 50% contraction (pEC$_{50}$) or 50% relaxation (pIC$_{50}$) was calculated for each individual concentration–response curve, and the mean±SEM is reported. Statistical evaluation of the data was performed by Student’s t test for paired or unpaired observations. When data of more than two groups were compared, an analysis of variance (ANOVA) test was used and individual means were compared with Scheffe’s F test. Values were considered statistically significant at p<0.05.

Results

Characterization of the Endothelium

A monolayer of endothelial cells covered the luminal surface of the intact carotid arteries but not of balloon-injured vessels (data not shown). Acetylcholine (10$^{-6}$ M) and calcium ionophore A23187 (10$^{-6}$ M) relaxed control carotid arteries with endothelium contracted with phenylephrine (10$^{-6}$ M) (Figure 1). Neither relaxant agent had any effect in control carotid arteries without endothelium or balloon-injured carotid arteries (24 hours) contracted with phenylephrine (10$^{-7}$ M; Figure 1).

Organ Chamber Studies

Phenylephrine evoked concentration-dependent contractions in control carotid arteries with and without endothelium. The presence of the endothelium significantly inhibited the response to phenylephrine (maximal contraction, 1.07±0.18 g and 1.73±0.24 g; pEC$_{50}$ 7.14±0.1 and 7.95±0.13 in rings with and without endothelium, respectively; n=5–6). The addition of NLA (10$^{-4}$ M) or SOD (100 IU/ml) to control carotid rings without endothelium did not significantly affect the basal tension or the concentration–contraction curve to phenylephrine (10$^{-7}$–10$^{-5}$ M; Table 1). Similar observations were obtained in control right carotid arteries without endothelium collected immediately after or 6 or 24 hours following in vivo balloon catheterization of the left carotid artery. In control carotid arteries with endothelium, NLA (10$^{-4}$ M) shifted the concentration–contraction curves to phenylephrine to the left and significantly increased the maximal contraction (from 1.07±0.18 to 2.04±0.16 g; n=5).

In the balloon-injured left carotid arteries, phenylephrine evoked concentration-dependent contractions. The concentration–contraction curves in rings collected 6 or 24 hours after injury were shifted significantly to the right of those obtained in rings collected immediately after the procedure (pEC$_{50}$ 7.48±0.07 and 7.33±0.13 compared with 7.93±0.09, respectively; Figure 2) or in the control right carotid artery without endothelium.

<table>
<thead>
<tr>
<th>Time (hrs)</th>
<th>$E_{max}$ (g)</th>
<th>pEC$_{50}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solvent</td>
<td>1.73±0.24</td>
<td>7.95±0.13</td>
</tr>
<tr>
<td>6</td>
<td>2.09±0.22</td>
<td>7.85±0.09</td>
</tr>
<tr>
<td>24</td>
<td>1.88±0.12</td>
<td>7.98±0.16</td>
</tr>
<tr>
<td>NLA (10$^{-4}$ M)</td>
<td>1.57±0.22</td>
<td>8.09±0.04</td>
</tr>
<tr>
<td>6</td>
<td>1.97±0.07</td>
<td>8.05±0.09</td>
</tr>
<tr>
<td>24</td>
<td>1.70±0.18</td>
<td>8.09±0.08</td>
</tr>
<tr>
<td>SOD (100 IU/ml)</td>
<td>1.95±0.27</td>
<td>7.81±0.05</td>
</tr>
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The results are presented as mean±SEM of six or seven different experiments and shown in absolute values.

All experiments were performed in the presence of indomethacin (10$^{-5}$ M). Control arteries were collected immediately and 6 or 24 hours after balloon injury of the contralateral arteries.

$E_{max}$, maximum contractile effect; pEC$_{50}$, negative logarithm of the effective molar concentration of phenylephrine causing 50% of the maximal contraction; NLA, nitro-l-arginine; SOD, superoxide dismutase.

![Figure 1](http://circres.ahajournals.org/)

**FIGURE 1.** Isometric tension recordings demonstrating the presence of endothelium-dependent relaxations to acetylcholine and calcium ionophore A23187 in carotid arteries with endothelium contracted with phenylephrine (10$^{-6}$ M) and the absence of relaxations to acetylcholine and calcium ionophore A23187 in carotid arteries without endothelium contracted with phenylephrine (10$^{-7}$ M) and in balloon-injured carotid arteries (24 hours after injury) contracted with phenylephrine (10$^{-7}$ M). Similar observations were made in three to 25 different experiments.

![Figure 2](http://circres.ahajournals.org/)

**FIGURE 2.** Concentration–contraction curves evoked by phenylephrine in balloon-injured carotid arteries collected immediately (t=0), 6, and 24 hours after injury. Experiments were performed in the presence of indomethacin (10$^{-5}$ M). Results are presented as mean±SEM of five to six different experiments and are shown in absolute values.
Results

carotid arteries

Phenylephrine

significantly

did not affect the basal tension in the balloon-injured carotid rings. The contractions to phenylephrine were not affected in the presence of NLA or SOD in the left carotid arteries injured immediately before experiments (Figure 3A). In carotid arteries injured 6 hours before experiments, SOD (100 IU/ml) but not NLA significantly reduced the concentration-dependent contractions evoked by phenylephrine (Figure 3B). The maximal contraction was decreased from 1.9±0.18 to 1.52±0.01 g and pEC_{50} was decreased from 7.48±0.07 to 7.20±0.09 (n=4–5) in the absence and presence of SOD, respectively (Figure 3B). In left carotid arteries injured 24 hours before experiments, the contractions evoked by phenylephrine were potentiated significantly by NLA (10^{-4} M), but the maximal contraction was not affected (pEC_{50}, 7.49±0.13 and 7.95±0.23, n=6, in the absence and presence of NLA, respectively; Figure 4A). l-Arginine (10^{-4} M) did not affect the contractions evoked by phenylephrine, but the amino acid prevented the potentiating effect of NLA (Figure 4A). SOD (100 IU/ml) shifted the concentration–response curve to phenylephrine significantly to the right and significantly decreased the maximal contractions (from 1.76±0.13 to 1.49±0.16 g, n=7–8; Figure 4B). The inhibitory effect of SOD on the contractions evoked by phenylephrine was potentiated, but not significantly, by l-arginine (10^{-4} M; Figure 4B). Methylene blue (10^{-5} M) shifted the concentration–con- traction curves to phenylephrine to the left without significantly affecting the maximal response in balloon-injured carotid rings (pEC_{50}, 7.33±0.13 and 7.64±0.11, n=8, in the absence and presence of methylene blue, respectively; Figure 4B).

The incubation of control right carotid arteries without endothelium for 4 hours in culture medium containing IL-1 β (30 IU/ml) shifted the concentration–con- traction curves to phenylephrine significantly to the right and reduced the maximal response from 1.98±0.17 to 1.05±0.14 g (n=7–8). In the presence of NLA (10^{-4} M), the contractions to phenylephrine were similar to those obtained in control right carotid arteries without endothelium (Figure 5A).

The incubation of left carotid arteries that had been injured by balloon catheterization 24 hours before the experiments for 4 hours in culture medium containing IL-1 β (30 IU/ml) shifted the concentration–con- traction curves to phenylephrine significantly to the right and reduced the maximal response to the α_{1}-adrenergic
agonist from 1.77±0.09 to 0.87±0.17 g (n=7-8). NLA (10⁻⁴ M) shifted the concentration–contraction curves to phenylephrine significantly to the left and increased the maximal contraction to 2.03±0.15 g. Furthermore, in the presence of NLA the contractions to phenylephrine were greater than those obtained in balloon-injured arteries and were similar to those obtained in the control carotid without endothelium (Figures 5A and 5B). L-Arginine (10⁻⁴ M) partially reversed the increase in tension evoked by NLA in IL-1 β-treated rings (data not shown). The pEC⁵₀ was significantly smaller in balloon-injured rings than in control rings without endothelium treated with IL-1 β (6.24±0.03 and 6.56±0.07, n=7-8, respectively).

SIN-1 evoked concentration-dependent relaxations in balloon-injured carotid rings (24 hours after injury) and in control carotid rings with and without endothelium (Figure 6). The pIC⁵₀ was similar in the three types of preparations (6.05±0.08, 5.95±0.06, and 5.81±0.13, n=8, respectively). Methylene blue (10⁻⁵ M) inhibited the relaxations evoked by SIN-1 in carotid arteries without endothelium as well as in balloon-injured arteries (Figure 6).

Production of cGMP

Incubation of right carotid rings without endothelium in the presence of NLA (10⁻⁴ M) did not significantly affect the content of cGMP (0.25±0.03 and 0.41±0.13 pmol/mg protein, n=4-5, in the absence and presence of NLA, respectively; Figure 7). In the balloon-injured arteries (24 hours after injury) the basal content of cGMP was significantly higher than that in control right carotid arteries without endothelium (2.07±0.7 and 0.25±0.03 pmol/mg protein, n=4-5, respectively; Figure 7). Incubation of balloon-injured carotid artery (24 hours after injury) in the presence of NLA significantly reduced the basal content of cGMP, from 2.07±0.7 to 0.47±0.04 pmol/mg protein (n=5; Figure 7).

The incubation of right carotid arteries without endothelium with IL-1 β (30 IU/ml for 4 hours) increased the tissue content of cGMP 13-fold, from 0.25±0.03 to 3.27±0.64 pmol/mg protein (n=4-5; Figure 7). Treatment of rings with NLA (10⁻⁴ M) inhibited the production of cGMP evoked by IL-1 β (Figure 7).

The incubation of balloon-injured left carotid arteries (24 hours after injury) with IL-1 β (30 IU/ml for 4 hours) increased the tissue content of cGMP fourfold, from 2.07±0.7 to 8.81±1.24 pmol/mg protein (n=5; Figure 7). The content of cGMP in IL-1 β–treated preparations was significantly higher in balloon-injured arteries than in control right carotid arteries without endothelium (8.81±1.24 pmol/mg protein compared with 3.27±0.64 pmol/mg protein; n=5). Treatment of rings with NLA (10⁻⁴ M) inhibited the production of cGMP caused by IL-1 β (Figure 7).

Discussion

The present study demonstrates that nitric oxide synthase activity is induced in the rat carotid artery following in vivo injury by balloon catheterization. The nitric oxide synthase activity results in a reduced reac-
tivity of the blood vessel to phenylephrine and is associated with an accumulation of cGMP.

In the rat carotid artery, the removal of the endothelium either by balloon catheter or mechanically by a small metal wire results in an increase of the contractions evoked by phenylephrine. The endothelium-mediated depression of the response is likely due to the basal release of endothelium-derived nitric oxide by the endothelial cells. In agreement with this interpretation, the removal of the endothelium in the rat thoracic aorta increases contractions induced by α-adrenergic agonists and is associated with a decreased production of cGMP. However, the destruction of the endothelium induces a smaller increase of the contractions to phenylephrine in carotid arteries collected 24 hours after injury than in control right carotid arteries without endothelium when compared with intact vessels. This effect is associated with an increased tissue content of cGMP. Furthermore, treatment of balloon-injured arteries collected 24 hours after injury with NLA, an inhibitor of nitric oxide synthesis, restores the contractile response to phenylephrine and the production of cGMP to a similar level as that in the control right carotid arteries without endothelium. These observations, in conjunction with the fact that methylene blue, an inhibitor of soluble guanylate cyclase, restores the contractility of the balloon-injured arteries, suggest that the in vivo endothelial injury by balloon catheterization induces the generation of a relaxing factor from l-arginine. The observation that SOD, which prevents the degradation of nitric oxide by superoxide anions, potentiates the reduced contractions to phenylephrine further supports the assumption that a sustained activation of the l-arginine–nitric oxide pathway accounts for the hyporeactivity to phenylephrine and the elevated tissue content of cGMP following balloon injury of the carotid artery. A decreased contractility to phenylephrine was detected as early as 6 hours after the in vivo injury procedure in the presence of SOD. This observation suggests that the amount of released nitric oxide by the vessel wall is not sufficient at this time to overcome its degradation by superoxide anions. The release of superoxide anions by the blood vessel wall may be due to the presence of neutrophils and macrophages.

l-Arginine did not affect the contractions to phenylephrine in balloon-injured carotid arteries, suggesting that the endogenous concentration of l-arginine is sufficient for the production of nitric oxide. In accordance with these results, l-arginine did not affect the contractions evoked by phenylephrine in freshly collected rat aortas. However, when the degradation of nitric oxide by superoxide anions was prevented by SOD, l-arginine slightly potentiated the inhibitory effect of balloon injury on vascular contractility. Thus, under these experimental conditions, the production of nitric oxide by the damaged blood vessel may be partially dependent on extracellular l-arginine.

The presence of an l-arginine–nitric oxide pathway in the balloon-injured carotid arteries cannot be explained by the presence of remaining endothelial cells following balloon catheterization, because acetylcholine and the calcium ionophore A23187 did not evoke relaxations, and histological studies did not demonstrate the presence of endothelial cells. Moreover, the impaired reactivity to phenylephrine was not observed immediately but appeared 24 hours after the in vivo injury. The possibility that the injury with a balloon catheter damaged the medial smooth muscle cells or affected the soluble guanylate cyclase–cGMP effector pathway is unlikely because phenylephrine evoked similar maximal contractions, and SIN-1, a donor of nitric oxide, evoked similar relaxations in balloon-injured and in control carotid arteries. Furthermore, methylene blue inhibited the relaxations evoked by SIN-1 to a similar extent in both preparations.

The stimulation of isolated blood vessels for several hours with cytokines such as IL-1 β or endotoxin reduces their responsiveness to vasoconstrictors in an endothelium-independent manner. This decreased vascular reactivity is associated with an increased production of cGMP and is prevented by inhibitors of nitric oxide synthesis. The treatment of cultured vascular smooth muscle cells with IL-1 β induces the release of a potent relaxing factor and stimulates the production of cGMP and the accumulation of nitrite, an oxidation product of nitric oxide, in the culture medium. The cytosol of cytokine-treated but not control smooth muscle cells activates soluble guanylate cyclase. These observations indicate that cytokines in-

![Figure 7](http://circres.ahajournals.org/attachment/10.1161/01.CIR.71.2.336.Figure7)
duce nitric oxide synthase(s) activity in vascular smooth muscle cells, which may contribute to the impaired reactivity of isolated blood vessels and to the inhibition of platelet-activation by cytokine-activated vascular smooth muscle cells. The treatment of carotid arteries with IL-1 β reduces the contractions to phenylephrine and stimulates the production of cGMP. Both responses are prevented by NLA. These observations indicate that IL-1 β, a mediator released from blood cells at sites of vascular injury, induces nitric oxide synthase activity in the carotid artery. The reduced contractility and the production of cGMP evoked by IL-1 β are larger in balloon-injured arteries that have been collected 24 hours after balloon injury than in control right carotid arteries without endothelium. Furthermore, in the presence of NLA the contractions to phenylephrine in balloon-injured arteries are greater than those obtained in the absence of IL-1 β and are similar to those obtained in control carotid arteries without endothelium. These observations support the hypothesis that vascular injury by balloon catheterization is associated with the induction of nitric oxide synthase activity in the blood vessel wall, even in the absence of the endothelium. The cell types involved in the production of nitric oxide in the injured carotid artery are unknown. However, potential candidates such as neutrophils, macrophages, platelets, smooth muscle cells, and fibroblasts could contribute. However, the most likely cells responsible for the generation of the relaxing factor are the vascular smooth muscle cells, since they are the most prominent cell type. Monocytes, macrophages, and other cells, when activated, produce factors such as IL-1 β that contribute to immune and inflammatory responses. The present results suggest that vascular smooth muscle contains a system that generates nitric oxide from L-arginine in vascular smooth muscle and that this system is activated following vascular injury in vivo. The activation may be due to cytokines released locally by blood cells adhering to the injured area. The functional importance of this pathway in regard to intimal hyperplasia following vascular injury is unknown. However, the capacity of vascular smooth muscle to produce nitric oxide following vascular injury may play an important role in inhibiting platelet adhesion and aggregation as well as adhesion of neutrophils. These events appear early after vascular injury. Thus, the production of nitric oxide by the vessel wall may help to prevent the activation of blood cells and the release of mitogenic factors such as platelet-derived growth factor, epidermal growth factor, and transforming growth factor-β. These peptides are capable of stimulating growth-related metabolism as well as inhibiting the induction of nitric oxide synthase by cytokines. The results of the present study suggest that nitric oxide participates in inflammatory responses following vascular injury. IL-1 β may be the mediator of the in vivo induction of nitric oxide synthase in the vessel wall following balloon angioplasty.

Acknowledgments
The authors wish to thank Barnabas Desta and Dewayne O. Coney for technical assistance.

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Circ Res. 1992;71:331-338
doi: 10.1161/01.RES.71.2.331

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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