Vascular Responsiveness to Nitric Oxide Synthesis Inhibition in Hypertensive Rats

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Abstract We contrasted in normotensive and hypertensive rats the effect of inhibition of nitric oxide synthesis on isometric tension development by aortic rings bathed in Krebs' bicarbonate buffer. \textsuperscript{N}^\text{O}-Nitro-L-arginine methyl ester (L-NAME) (3x10\textsuperscript{-4} mol/L) increased tension by 82±11% of the response to 120 mmol/L potassium chloride in rings of thoracic aorta taken from hypertensive rats 7 to 14 days after aortic coarctation, whereas rings of abdominal aorta from below the coarctation were unresponsive, as were rings of thoracic aorta from rats with deoxycorticosterone-salt-induced hypertension and from the corresponding normotensive controls of either model of hypertension. The contractile response to L-NAME in aortic rings of rats with aortic coarctation was reversed by L-arginine (1 mmol/L), attenuated by removal of the endothelium, and blunted by the protein kinase C inhibitor staurosporine but was unaffected by inhibition of cyclooxygenase, scavengers of superoxide anion, or blockade of receptors for angiotensin, norepinephrine, serotonin, or endothelin. In additional experiments we contrasted the effect of L-NAME (10 mg/kg IV) on the blood pressure of sham-operated rats and rats with aortic coarctation after pretreatment of animals in both groups with DuP 753 (30 mg/kg IV) to achieve blood pressure equalization. The pressor response to L-NAME was twofold greater in rats with aortic coarctation than in sham-operated controls. That pressor and aortic constrictor responsiveness to L-NAME are increased after aortic coarctation suggests that a mechanism of vasodilation, mediated by nitric oxide, is preferentially manifested in rats with aortic coarctation-induced hypertension.

Nitric oxide (NO) relaxes vascular smooth muscle by increasing the formation of cyclic GMP via activation of a soluble guanylate cyclase.\textsuperscript{1} Blood vessels manufacture NO from the amino acid L-arginine.\textsuperscript{2,3} Endothelial cells are the primary site of vascular NO synthesis,\textsuperscript{4,5} which is catalyzed by a constitutive enzyme both under basal conditions and during stimulation by various vasoactive hormones.\textsuperscript{5} Vascular smooth muscle cells and endothelial cells also are capable of synthesizing NO after exposure to endotoxin or cytokines that induce an NO synthase different from the constitutive enzyme found in endothelial cells.\textsuperscript{6,7}

Treatment with inhibitors of NO syntheses causes elevation of blood pressure due to vasoconstriction in multiple vascular beds,\textsuperscript{8,9} increases vasoconstrictor responsiveness to angiotensin II (Ang II) and other vasoconstrictor agonists,\textsuperscript{10} and decreases vasodilator responsiveness to acetylcholine, bradykinin, and other endothelin-dependent vasodilator agents.\textsuperscript{11} Such responses to treatment with NO synthesis inhibitors imply that endogenous NO subserves important mechanisms of circulatory control.

Reports that endothelium-dependent vasorelaxant responses to acetylcholine are diminished in various models of experimental hypertension have fostered the notion that vasodilator mechanisms mediated by NO are impaired in hypertension.\textsuperscript{12} A similar conclusion was derived from observations that inhibitors of NO synthesis have greater pressor and vasoconstrictor effects in salt-resistant normotensive rats than in salt-sensitive rats made hypertensive by high salt intake.\textsuperscript{13} Contrary to the notion that NO-mediated vasodilator mechanisms are impaired in hypertension, there are reports that NO release from isolated perfused aortas is similar in normotensive and hypertensive rats\textsuperscript{14} and that pressor and vasoconstrictor responses to treatment with inhibitors of NO synthesis are similar in normotensive and spontaneously hypertensive rats.\textsuperscript{15}

Recently, while investigating the influence of NO on constrictor responses to arachidonic acid in rings of thoracic aorta taken from rats with aortic coarctation-induced hypertension, we found that aortic rings taken from hypertensive but not from normotensive rats contract when challenged with an inhibitor of NO synthesis. The studies reported in this article aim at characterizing the differential effect of inhibitors of NO synthesis on isometric tension development by rings of descending thoracic aorta taken from sham-operated normotensive rats and rats with aortic coarctation-induced hypertension. We also compared the blood pressure • muscle, smooth, vascular effect of cyclooxygenase, scavengers of superoxide anion, or blockade of receptors for angiotensin, norepinephrine, serotonin, or endothelin. In additional experiments we contrasted the effect of L-NAME (10 mg/kg IV) on the blood pressure of sham-operated rats and rats with aortic coarctation after pretreatment of animals in both groups with DuP 753 (30 mg/kg IV) to achieve blood pressure equalization. The pressor response to L-NAME was twofold greater in rats with aortic coarctation than in sham-operated controls. That pressor and aortic constrictor responsiveness to L-NAME are increased after aortic coarctation suggests that a mechanism of vasodilation, mediated by nitric oxide, is preferentially manifested in rats with aortic coarctation-induced hypertension. (Hypertension. 1994;23(part 1):744-751.)

Key Words • nitric oxide • vascular resistance • blood pressure • muscle, smooth, vascular
wise noted, all other drugs and chemicals were obtained from Sigma Chemical Co.

Pyrogen-free, deionized water was used in the preparation of all buffers and solutions. Stock solutions of phenylephrine, acetylcholine, DuP 753, phosphoramidon, 4,5-dihydroxy-1,3-benzenedisulfonic acid (tiron), and phentolamine were prepared in deionized water; of ketanserin in 2.34 mmol/L tartaric acid; of PED3512 in 1.0 mol/L NaHCO3; and of staurosporine in 50 mmol/L Na2CO3; A^-nitro-L-arginine methyl ester (L-NAME), N^2-nitro-D-arginine methyl ester (D-NAME), and L-NMMA were prepared by dissolution in deionized water. The composition of Krebs' bicarbonate buffer (37°C) bubbled with 95% O2-5% CO2 and 3-isobutyl-1-methylxanthine (IBMX) in dimethyl sulfoxide. Indomethacin was prepared at the time of use by dissolution in 50 mmol/L Na2CO3; N^2-nitro-L-arginine methyl ester (L-NAME), N^2-nitro-D-arginine methyl ester (D-NAME), and L-NMMA were prepared by dissolution in deionized water. The composition of Krebs' bicarbonate buffer (mmol/L) was NaCl 118.5, KCl 4.7, CaCl2 2.8, KH2PO4 1.2, MgSO4 1.1, NaHCO3 25.0, and dextrose 11.1.

Animals

Procedures described below were approved by the Institutional Animal Care and Use Committee. Experiments were conducted on male Sprague-Dawley rats (Charles River) with aortic coarctation–induced hypertension, deoxytococortesterone acetate (DOCA)–salt–induced hypertension, and Ang II–induced hypertension and in the respective sham-operated and vehicle-treated normotensive control rats. Rats (300 to 325 g) with aortic coarctation or sham aortic coarctation were prepared according to a published procedure. Brieﬂy, after induction of anesthesia with pentobarbital sodium (60 mg/kg IP), the abdominal aorta was completely ligated at a point below the right but above the left renal artery. Experiments were conducted 7 to 14 days and 28 to 42 days after aortic coarctation or sham surgery. Rats (175 to 200 g) with DOCA-salt–induced hypertension and the corresponding normotensive controls were prepared as follows. After rats were anesthetized with methoxyflurane (Pitman-Moore, Inc), the left kidney was removed. Thereafter, the animals received subcutaneous injections twice weekly of DOCA (20 mg/kg in sesame oil) or sesame oil only (1 ml/kg) and were given a saline solution (NaCl, 1.0 g/100 mL) to drink until experiments were performed 28 to 42 days later. Rats with Ang II–induced hypertension (300 to 325 g) were prepared by administration of Ang II (200 ng/min) by means of an osmotic minipump (model 2001, Alza Corp) placed in the tissue bath 15 to 20 minutes before the addition of L-NAME or methylene blue. Experiments were conducted 7 days after minipump placement.

Effects of Inhibitors of NO Synthesis on Isometric Tension of Aortic Rings From Normotensive and Hypertensive Rats

The right carotid artery, and in some experiments the femoral artery, of rats anesthetized with pentobarbital sodium (60 mg/kg IP) was cannulated with polyethylene tubing (PE-50) connected to a pressure transducer (model P23ID, Statham) for recording of mean arterial pressure on a polygraph (model 7D, Grass Instrument Co). After blood pressure measurement the descending thoracic aorta, the abdominal aorta distal to the left renal artery, or both were excised, transferred to a dish filled with ice-cold Krebs' bicarbonate buffer, and cut transversely into ring segments (5.0 mm in length). In some experiments the luminal surface of aortic rings was rubbed with a small piece of polyethylene tubing to remove the endothelium.

Each aortic ring was placed in a tissue bath filled with Krebs' bicarbonate buffer (37°C) bubbled with 95% O2-5% CO2 and was attached to a force-displacement transducer (model FT03C, Grass) coupled to a polygraph (model 7D, Grass) for measurement of isometric tension as previously described. Rings were allowed to equilibrate for 60 to 90 minutes at a resting tension of 2.0 g with changes of buffer at 15-minute intervals. In preliminary experiments we found that 2.0 g of resting tension is optimal for the expression of potassium chloride (KCl)–induced contraction of aortic rings obtained from normotensive and hypertensive rats.

Experiments were initiated by obtaining a reference contractile response to KCl (120 mmol/L). After rinsing, the presence or absence of functional endothelium was tested by assessing the ability of acetylcholine (10^-4 mol/L) to relax aortic rings precontracted with phenylephrine (5x10^-4 to 5 x10^-3 mol/L). Subsequently, aortic rings were rinsed, and once tension had returned to baseline, L-NAME (3x10^-4 to 2x10^-3 mol/L) or L-NMMA (3x10^-4 mol/L), which inhibit NO synthesis, and D-NAME (3x10^-4 mol/L), which is without effect on NO synthesis, or methylene blue (10^-3 mol/L), which inhibits soluble guanylate cyclase, was added to the tissue bath, and the isometric tension was monitored for 60 minutes or until a plateau response was obtained. In some experiments, once the contractile response to L-NAME or L-NMMA (3x10^-4 mol/L) was obtained, the effect of subsequent addition of D-arginine (1 mol/L) or L-arginine (10^-4 mol/L) was recorded. In other experiments the effect of L-NAME (3x10^-4 mol/L) on tension development by rings of thoracic aorta from rats with aortic coarctation–induced hypertension was studied in the absence and presence of cycloheximide (2x10^-5 mol/L) or dexamethasone (10^-5 mol/L), which were added to the tissue bath at the onset of the equilibration period to prevent induction of vascular NO synthases in the ex vivo setting.

The effects of L-NAME and methylene blue on the tone of rings of descending thoracic aorta taken from rats with aortic coarctation–induced hypertension were studied also in the absence and presence of agents known to interfere with the activity of various endogenous vasococontracting systems. Indomethacin (10^-4 mol/L) was used to inhibit synthesis of vasoconstrictor prostanoids. DuP 753 (10^-4 mol/L) to block Ang II type 1 (AT1) receptors, phenylephrine (10^-4 mol/L) to block a-adrenergic receptors, ketanserin (10^-5 mol/L) to block type 2 serotonin (5-HT2) receptors, phosphoramidon (10^-4 mol/L) to inhibit the conversion of "big" endothelin to endothelin-1, PED3512 (10^-5 mol/L) to block endothelin-A (ET-A) receptors, superoxide dismutase (50 U/mL) or tiron (10^-2 mol/L) to scavenge superoxide anions, and staurosporine (10^-4 mol/L) to inhibit protein kinase C activity. The interfering agents or the corresponding vehicle were added to the tissue bath 15 to 20 minutes before the addition of L-NAME (3x10^-4 mol/L) or methylene blue (10^-3 mol/L), except staurosporine, which was added to the bath 60 to 120 minutes before L-NAME. After the interfering agents, the ensuing changes in isometric tension were monitored until the response reached a plateau. The interfering agents referred to above were used in concentrations shown to be effective by previous studies.

For agents that elicit relaxation of aortic rings preconstricted by a drug, the response is expressed as the percentage reduction of tension in the preconstricted state. For agents that elicit constriction of aortic rings, the response is expressed as the percentage of the reference contractile response to KCl (120 mmol/L). The increase in isometric tension produced by KCl (120 mmol/L) in vascular rings of sham-operated rats and rats with aortic coarctation–induced hypertension was, respectively, 2.66±0.30 and 1.77±0.26 g (P<.05) for the abdominal aorta below the coarctation and 2.59±0.31 and 1.84±0.12 g (P<.05) for the descending thoracic aorta. KCl (120 mmol/L) increased the tension of rings of descending thoracic aorta taken from normotensive controls and rats with DOCA-salt–induced hypertension by 2.70±0.15 and 3.00±0.19 g, respectively.

Complementary experiments compared the activity of NO synthase in normal and aortic tissue from rats with aortic coarctation–induced hypertension and sham-operated normotensive controls. NO synthase activity was measured according to a published procedure, with some modifications. Descending thoracic aortas of rats subjected to aortic coarct-
tation or sham surgery 7 to 14 days previously were excised, pooled (three aortas per assay), and homogenized in ice-cold Tris-HCl (50 mM, pH 7.5) assay buffer. After centrifugation (2500 ×g for 30 minutes) endogenous L-arginine was removed from the supernatant by passage over a column containing Dowex AG 50W-X8 cation-exchange resin (Na+ form) (Bio-Rad), and glycerin (10% vol/vol) was added to the eluate. Aliquots of column eluate (0.18 mL, 100 to 200 μg protein) were incubated with L-arginine HC1 (10–4 M) and [3H]-L-arginine (0.2 μCi; specific activity, 57 Ci/mmol), nicotinamide adenine dinucleotide phosphate (10–3 M), tetrahydrobiopterin (3 × 10–6 M), calmodulin (10–7 M), and EGTA and EDTA (both 2 mM) for 10 minutes. Reactions were then terminated by addition of 1 mL of cold HEPES buffer (20 mM, pH 5.5) containing 10–3 M indomethacin to inhibit cyclooxygenase and to remove unreacted L-arginine. Radioactive citrulline in the column eluate was determined by liquid scintillation counting.

cGMP content of rings of descending thoracic aorta of rats with aortic coarctation or sham surgery was measured by a previously published procedure,28 with some modifications. Aortic rings were snap-frozen in liquid nitrogen after incubation for 1 hour in Krebs’ bicarbonate buffer with IBMX (0.5 mM) and subsequently homogenized in ice-cold trichloroacetic acid (10 g/100 mL). Homogenates were extracted with diethyl ether (4 vol, three times), and the aqueous phase was evaporated under vacuum. After reconstitution with deionized water, cGMP was measured by radioimmunoassay (Advanced Magnetics).

Effect of L-NAME on Blood Pressure of Sham-Operated Rats and Rats With Aortic Coarctation

Rats subjected to aortic coarctation or sham surgery 7 to 14 days earlier were anesthetized with methohexital. The right external jugular vein and right carotid artery were cannulated with polyethylene tubing (PE-50), and the arterial cannula was connected to a pressure transducer for recording of blood pressure on a polygraph (instrument as above). After a 60-minute period to allow rats to wake up and stabilize, DuP 753 (30 mg/kg IV bolus) was administered to animals in both groups. After another 30-minute period either L-NAME (10 mg/kg IV bolus) or phenylephrine (4 μg · kg−1 · min−1 IV) was administered and blood pressure recorded until the response reached a plateau. DuP 753 is expected to normalize the blood pressure of rats in the early phase of aortic coarctation-induced hypertension 7 to 14 days after coarctation while having little or no hypotensive effect in sham-operated normotensive rats. Accordingly, DuP 753 may be expected to facilitate the comparison of blood pressure responsiveness to L-NAME and phenylephrine in such groups of rats.

Statistical Analysis

Results are expressed as mean ± SEM; n indicates the number of observations. Single-variable comparisons were made with a paired or unpaired Student’s t test, and all other data were analyzed by one- or two-way ANOVA, as appropriate. If differences were noted, Newman-Keuls modified t test was used to make specific comparisons. The null hypothesis was rejected when the probability value was less than .05.

Results

The mean carotid arterial pressure of rats 7 to 14 days (168 ± 5 mm Hg) and 28 to 42 days (166 ± 7 mm Hg) after aortic coarctation exceeded (P < .01) that of their corresponding sham-operated controls (113 ± 3 and 130 ± 13 mm Hg, respectively). The blood pressure measured in the carotid artery (164 ± 4 mm Hg) of rats 12 days after aortic coarctation also exceeded that measured simultaneously below the coarctation (80 ± 6 mm Hg) via a cannula in a femoral artery. The mean carotid arterial pressure of saline-drinking rats treated with DOCA for 28 to 42 days (166 ± 9 mm Hg) and of rats infused with Ang II for 7 days (178 ± 6 mm Hg) surpassed (P < .01) that of rats treated with the corresponding drug vehicle only (101 ± 6 and 105 ± 4 mm Hg, respectively).

Fig 1 depicts the effect of L-NAME, L-NMMA, and D-NAME (all at 3 × 10–4 mol/L) on isometric tension development by rings of descending thoracic aorta taken from hypertensive rats at 7 to 14 days after aortic coarctation and from sham-operated normotensive rats. Both unrubbed and rubbed rings of thoracic aorta from hypertensive rats were contracted by L-NAME and L-NMMA, but the response of rubbed rings was less prominent (P < .05) than that of unrubbed rings. Increases in tension produced by L-NAME in unrubbed rings from hypertensive rats averaged 3 ± 2%, 17 ± 8%, 48 ± 14%, 73 ± 5%, and 77 ± 4% of KCl response at 3 × 10–5, 3 × 10–4, 10–3, 3 × 10–4, and 10–2 mol/L, respectively (n = 4–10). In the same range of concentrations, mehcalon, a product of L-NAME hydrolysis by tissue esterases, was without constrictor effect on aortic rings of hypertensive rats (n = 4). D-NAME (3 × 10–4 mol/L) did not affect the tone of unrubbed rings but contracted rubbed aortic rings taken from hypertensive rats. Neither L-NAME nor L-NMMA or D-NAME (each at 3 × 10–4 mol/L) affected the tone of rings of thoracic aorta taken from normotensive rats. Moreover, L-NAME (3 × 10–4 mol/L) did not promote tension development in either rings of abdominal aorta taken from sham-operated rats (n = 7) or rats with aortic coarctation of 7 to 14 days’ duration (n = 6), rings of descending
thoracic aorta taken from sham-operated normotensive rats (n=4) or hypertensive rats at 28 to 42 days after aortic coarctation (n=10), or rings of descending thoracic aorta taken from rats with DOCA-salt-induced hypertension (n=16) or from the corresponding sesame oil-treated normotensive controls (n=11). On the other hand, L-NAME (3x10^-4 mol/L) did promote tension development in unrubbed rings of thoracic aorta (55±15% of KCl response, n=7) and rings of abdominal aorta (66±5% of KCl response, n=4) taken from rats with Ang II-induced hypertension but not from normotensive controls.

In some experiments L-NAME (3x10^-4 mol/L) was administered to unrubbed rings of thoracic aorta from rats 7 to 14 days after aortic coarctation without prior exposure to KCl. In these preparations L-NAME-induced contractions (1.35±0.03 g tension) were similar to those in rings preexposed to KCl (1.45±0.40 g tension). The constrictor effect of L-NAME (3x10^-4 mol/L) in rings of thoracic aorta from hypertensive rats at 7 to 14 days after coarctation was similar in the absence and presence of either cycloheximide (2x10^-4 mol/L; 53±6% versus 78±14% of KCl response; n=5 and 8, respectively) or dexamethasone (10^-7 mol/L; 53±6% versus 78±14% of KCl response; n=5 and 7, respectively), added to the tissue bath during the equilibration period to prevent induction of NO synthase in the ex vivo setting.

As shown in Fig 2, L-arginine (1 mmol/L) added to the tissue bath caused rapid reversal of the contraction induced by L-NAME (-96±6% tone change, n=18) and L-NMMA (-108±9% tone change, n=8) in unrubbed rings of thoracic aorta taken from hypertensive rats at 7 to 14 days after aortic coarctation. However, L-arginine (1 mmol/L) was less effective in reversing the contraction induced by either L-NAME (-30±8% tone change, n=17) or L-NMMA (-26±4% tone change, n=8) in rubbed aortic rings of hypertensive rats. Contractions induced by D-NAME (3x10^-4 mol/L) in rubbed aortic rings from hypertensive rats were unaffected by L-arginine (-3±11% tone change, n=4). D-Arginine was without effect on the contractions induced by L-NAME (+5±3% tone change, n=9).

NO synthase activity, as estimated by the conversion of L- arginine to L-citrulline, was similar in homogenates of descending thoracic aorta of sham-operated normotensive rats (35.6±12.6 pmol/mg protein^-1·10^-3 min^-1, n=4) and hypertensive rats at 7 to 14 days after aortic coarctation (40.7±5.3 pmol/mg protein^-1·10^-3 min^-1, n=4). Similarly, rings of descending thoracic aorta taken from sham-operated normotensive rats and hypertensive rats at 7 to 14 days after aortic coarctation had similar levels of cGMP (9.2±3.6 and 8.5±2.4 pmol/mg protein^-1·10^-3 min^-1, n=4 and 6, respectively).

Fig 3 contrasts, in unrubbed rings of descending thoracic aorta taken from hypertensive rats at 7 to 14 days after aortic coarctation, the constrictor response to L-NAME (3x10^-4 mol/L) in the absence and presence of agents known to interfere with the synthesis or actions of various mediators of vascular contraction. The constrictor response in aortic rings to this inhibitor of NO synthesis was not affected by either inhibition of prostanooid synthesis with indomethacin (10^-5 mol/L), blockade of AT1 receptors with DuP 753 (10^-5 mol/L), blockade of a-adrenergic receptors with phentolamine (10^-5 mol/L), blockade of 5-HT2 receptors with ketanserin (10^-5 mol/L), inhibition of neutral endopeptidase 24.11 with phosphoramidon (10^-4 mol/L), or blockade of ETA receptors with PED3512 (10^-5 mol/L). Aortic constrictor responses to L-NAME also were similar in the absence and presence of superoxide dismutase (50 U/mL; 56±4% versus 53±13% of KCl response; n=7 and 6, respectively) or tiron (10^-7 mol/L; 59±2% versus 65±5% of KCl response; n=5 and 7, respectively), which each scavenge superoxide anion. However, as shown in Fig 4, constrictor responses to L-NAME in both unrubbed and rubbed aortic rings from hypertensive rats 7 to 14 days after aortic coarctation were blunted in rings preexposed to staurosporine (10^-7 mol/L).
Aortic Coarctation-Unrubbed
Aortic Coarctation-Rubbed
Sham-Unrubbed
Sham-Rubbed

Staurosporine (10^9 mol/L)
L-NAME (3X10^-10 mol/L)

0 60 120 180
TIME (min)

Fig 4. Representative tracings show isometric tension in unrubbed and rubbed rings of thoracic aorta from hypertensive rats 7 to 14 days after aortic coarctation and from normotensive, sham-operated controls sequentially exposed to staurosporine (10^9 mol/L) and N^2-nitro-L-arginine methyl ester (L-NAME, 3X10^-10 mol/L).

Fig 5 illustrates the effect of methylene blue, an inhibitor of soluble guanylate cyclase, on isometric tension development by rings of thoracic aorta taken from hypertensive rats at 7 to 14 days after aortic coarctation and from sham-operated normotensive rats. Methylene blue (10^-5 mol/L) did not affect the tone of aortic rings from normotensive rats but promoted tension development in aortic rings from hypertensive rats. Both unrubbed and rubbed aortic rings from hypertensive rats were contracted by methylene blue, but the response of rubbed rings was less prominent (P<.05) than that of unrubbed rings. The constrictor response to methylene blue in unrubbed aortic rings from hypertensive rats was similar in the absence (6±3% of KCl response, n=11 for unrubbed rings and 7±3% of KCl response, n=12 for rubbed rings). Importantly, staurosporine alone caused profound relaxation of both unrubbed (−1.10±0.15 g tension, n=14) and rubbed (−1.16±0.12 g tension, n=12) aortic rings taken from hypertensive rats 7 to 14 days after aortic coarctation but not from sham-operated normotensive rats (0.00±0.00 and −0.10±0.05 g tension, n=8 and 7, respectively).

Fig 6 illustrates the results of experiments in which the effect of either L-NAME (10 mg/kg IV bolus) or phenylephrine (4 μg·kg^-1·min^-1 IV) on blood pressure was contrasted in sham-operated rats and rats 7 to 14 days after aortic coarctation. Whereas DuP 753 did not cause a significant change in the mean arterial pressure of sham-operated rats, it did induce a large decrease in mean arterial pressure in rats with aortic coarctation-induced hypertension. After treatment with DuP 753 alone, blood pressure was similar in both groups. The subsequent administration of L-NAME caused a considerable increase in mean arterial pressure in both groups. However, the increase seen in rats with aortic coarctation was twice as large (P<.01) as that seen in the sham-operated animals. On the other hand, the increase in mean arterial pressure produced by the administration of phenylephrine was similar in sham-operated rats and rats 7 to 14 days after aortic coarctation.

Discussion

The central finding of this study is that L-NAME and L-NMMA caused contraction of rings of descending thoracic aorta taken from rats with aortic coarctation-induced hypertension of 7 to 14 days’ duration but not from sham-operated normotensive rats. That rubbing of the lumen resulted in attenuation but not in elimination of the constrictor effect of these agents in aortic rings of hypertensive rats suggests a dual contribution of endothelium-
were conditioned by ex vivo induction of NO synthases, because the constrictor response also could be unlikely that the constrictor responses to L-NAME and L-NMMA in endothelium-denuded rings from hypertensive rats that can be reversed with L-arginine. It is for that part of the constrictor effect of L-NAME and L-NMMA may be ascribed to diminished basal inhibition.

The synthesis of NO in normal arterial vessels is catalyzed by a constitutive, calcium-dependent NO synthase that is expressed in vascular smooth muscle. In the present study constrictor responses to L-NAME and L-NMMA in unrubbed rings of thoracic aorta from hypertensive rats 7 to 14 days after aortic coarctation were fully reversed by L-arginine, which is an impediment to the inhibitory effects of both constricting agents on NO synthases. Consequently, these constrictor responses to L-NAME and L-NMMA may be ascribed to diminished basal synthesis of NO. That constrictor responses to L-NAME and L-NMMA in rubbed aortic rings were only partially reversed by L-arginine suggests that in rings without endothelium part of the constrictor response to these agents is linked to inhibition of NO synthesis and part is not. As shown for D-NAME, removal of the endothelium may uncover a constrictor action of L-NAME and L-NMMA on aortic rings of hypertensive rats that is unrelated to NO synthesis inhibition.

dependent and endothelium-independent mechanisms to the constrictor response.

The expression of constrictor responses to L-NAME in rings of thoracic aorta from hypertensive rats at 7 to 14 days after aortic coarctation may be linked to conditions created by exposure of the vascular segment to increased blood pressure, because rings of abdominal aorta taken from below the site of coarctation (a vascular segment exposed to normal or somewhat reduced blood pressure) did not contract in response to L-NAME. Previous studies also demonstrated pressure dependency of functional and morphological changes in the aorta of rats with coarctation-induced hypertension. But in the present study L-NAME did not stimulate contraction of rings of thoracic aorta taken from hypertensive rats 28 to 42 days after aortic coarctation or from rats with DOCA-salt-induced hypertension. It may appear, then, that exposure of the aorta to increased blood pressure is necessary but not sufficient for expression of constrictor responsiveness to L-NAME. Perhaps hormonal abnormalities, ie, increased activity of the renin-angiotensin system, particular to rats in the early phase of aortic coarctation–induced hypertension, are also necessary. Relative to this point, the present study demonstrates that L-NAME contracts rings of descending thoracic aorta as well as rings of abdominal aorta taken from rats made hypertensive by Ang II infusion.

NO of vascular origin is a mediator of relaxation of Leukocytes and macrophages, reported to infiltrate the aorta to increased blood pressure is necessary but not sufficient for expression of constrictor responsiveness to L-NAME. Perhaps hormonal abnormalities, ie, increased activity of the renin-angiotensin system, particular to rats in the early phase of aortic coarctation–induced hypertension, are also necessary. Relative to this point, the present study demonstrates that L-NAME contracts rings of descending thoracic aorta as well as rings of abdominal aorta taken from rats made hypertensive by Ang II infusion.

Like L-NAME, methylene blue stimulates contraction of both unrubbed and rubbed rings of thoracic aorta taken from hypertensive rats with aortic coarctation of 7 to 14 days' duration but not from sham-operated controls. Moreover, the contractions induced by methylene blue were more prominent in unrubbed than rubbed aortic rings. Methylene blue inhibits soluble guanylate cyclase and attenuates vasodilator responses mediated by NO. Consequently, the constrictor response to methylene blue in aortic coarctation is attributable to inhibition of vascular cGMP synthesis, with attendant impairment in vasodilator responsiveness to endogenous NO.

That L-NAME, L-NMMA, and methylene blue stimulate contraction of rings of thoracic aorta taken from hypertensive rats 7 to 14 days after aortic coarctation but not from normotensive controls implies that the regulatory influence of endogenous NO on isometric tension development by the rings is unequally manifested in such animals. However, our study shows that both NO synthase activity and cGMP levels are similar in aortas taken from sham-operated normotensive rats and hypertensive rats 7 to 14 days after aortic coarctation. Thus, the disparity in the responsiveness of aortic smooth muscle from normotensive and hypertensive rats to both the inhibitors of NO synthesis and guanylate cyclase cannot be attributed to associated differences in the amount of NO or cGMP in aortic tissues. Rather, our results conform to those one would expect if the regulatory influence of equivalent amounts of NO produced by aortic rings of normotensive and hypertensive rats 7 to 14 days after aortic coarctation is more prominent in the aortic rings from hypertensive than from normotensive rats.

Basally produced NO acts as a physiological antagonist and/or inhibits the synthesis of vasoconstrictor hormones, including certain prostanoids, Ang II, norepinephrine, serotonin, endothelin, and superoxide anion. One or more of these hormones may be produced in excess by the arterial vessels of hypertensive animals. Therefore, constrictor responses to L-NAME and methylene blue in aortic rings from rats with aortic coarctation–induced hypertension may result from amplification of the actions of one or more vasoconstrictor hormones, consequent to inhibition of NO synthesis or actions. In examining this hypothesis we found that neither pretreatment with indomethacin nor DuP 753 or phenolamine caused attenuation of constrictor responses to L-NAME or methylene blue in aortic rings of rats with aortic coarctation of 7 to 14 days' duration. We also found that pretreatment with phosphoramidon, PED3512, superoxide dismutase, or tiron interfered with the constrictor response to L-NAME in aortic rings of hypertensive rats. Hence, our data do not support the notion that constrictor responses to L-NAME and methylene blue in aortic rings of rats with aortic coarctation–induced hypertension are the result of amplification of the vasoconstrictor actions of endogenous prostanoids, Ang II, norepinephrine, serotonin, endothelin, or superoxide anion. That the constrictor response to L-NAME and methylene demonstrated in rings pretreated with cycloheximide or dexamethasone, which prevent such an induction from occurring.
blue in aortic rings from hypertensive rats 7 to 14 days after aortic coarctation was blunted by staurosporine suggests dependency of the constrictor response on protein kinase C activity. As staurosporine decreased the resting tone of aortic rings taken from hypertensive but not from normotensive rats, it is conceivable that the constrictor responses to inhibitors of NO synthesis or actions in aortic rings of hypertensive rats relate to amplification of a preexistent tone that is dependent on protein kinase C activity. Reports that inhibitors of NO synthesis do not stimulate contraction of aortic rings from normal rats unless the rings are submaximally precontracted with an agonist also support the notion that expression of aortic smooth muscle response to NO synthesis inhibition is related to the preexistent tone.18

The observation that inhibition of NO synthesis with L-NAME impacts differently on the tone of vascular smooth muscle from sham-operated normotensive rats and rats with aortic coarctation of 7 to 14 days' duration may not be limited to the thoracic aorta only. In this regard we found that after equalization of blood pressure by pretreatment with DuP 753, the pressor response to L-NAME was greater in rats with aortic coarctation than in sham-operated controls. The acute increase in blood pressure produced by L-NAME is determined primarily by augmentation of peripheral vascular resistance due to inhibition of NO synthesis in resistance vessels.9 Accordingly, the increased pressor responsiveness to L-NAME in rats with aortic coarctation fits well with the notion that a mechanism of peripheral vasodilatation mediated by NO is manifested more prominently in rats with aortic coarctation than in sham-operated controls.

Acknowledgments
This work was supported by US Public Health Service grants HL-18579, HL-35670, and HL-34300.

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Hypertension. 1994;23:744-751
doi: 10.1161/01.HYP.23.6.744

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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