Differential inducible nitric oxide synthase expression in systemic and pulmonary vessels after endotoxin

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Pulido, Edward J., Brian D. Shames, David A. Fullerton, Brett C. Sheridan, Craig H. Selzman, Fabia Gamboni-Robertson, and Robert C. McIntyre, Jr. Differential inducible nitric oxide synthase (iNOS) expression in systemic and pulmonary vessels after endotoxin. Am J Physiol Regulatory Integrative Comp Physiol 278: R1232–R1239, 2000.—Inducible nitric oxide synthase (iNOS) is associated with vascular hypocontractility in systemic vessels after endotoxin lipopolysaccharide (LPS) administration. Although lung iNOS is increased after LPS, its role in the pulmonary circulation is unclear. We hypothesized that whereas iNOS upregulation is responsible for LPS-induced vascular dysfunction in systemic vessels, iNOS does not play a significant role in the pulmonary artery (PA). Using isolated aorta (AO) and PA rings, we examined the effect of nonselective NOS inhibition [N\(^\text{G}\)-monomethyl-L-arginine (L-NMMA); 100 \mu mol/l] and selective iNOS inhibition (aminoguanidine, AG; 100 \mu mol/l) on \(\alpha\)-adrenergic-mediated vasoconstriction (phenylephrine; 10\(^{-9}\) to 10\(^{-3}\) M) after LPS (Salmonella typhimurium, 20 mg/kg ip). We also determined the presence of iNOS using Western blot and immunohistochemistry. LPS markedly impaired AO contractility (maximal control tension 1,076 ± 33 mg vs. LPS 412 ± 39 mg, \(P < 0.05\)), but PA contractility was unchanged (control 466 ± 29 mg vs. LPS 455 ± 27 mg, \(P > 0.05\)). Selective iNOS inhibition restored the AO’s response to vasoconstriction (LPS + AG 1,135 ± 54 mg, \(P > 0.05\) vs. control and \(P < 0.05\) vs. LPS, but had no effect on the PA (LPS + AG 422 ± 38 mg, \(P > 0.05\) vs. control and LPS). Western blot and immunohistochemistry revealed increased iNOS expression in the AO after LPS, but iNOS was not detected in the PA. Our results suggest that differential iNOS expression after LPS in systemic and pulmonary vessels contributes to the phenomenon of sepsis/endotoxemia-induced systemic hypotension and pulmonary hypertension.

THE PREDOMINANT HEMODYNAMIC CHANGES in sepsis/endotoxemia are systemic hypotension and pulmonary hypertension (31, 33, 44). Abnormal regulation of vascular tone plays a role in the development of these contrasting events. Although endotoxin [lipopolysaccharide (LPS)] impairs cGMP-mediated relaxation in both the systemic and pulmonary circulations, the response to various forms of vasoconstriction is markedly diminished in the former but remains normal in the latter (7, 12, 22, 28, 38). These changes in vascular reactivity favor a decrease in vascular pressure and resistance in the systemic circulation and an increase in these parameters in the pulmonary circulation, thus helping to explain the paradoxical hemodynamic observations in sepsis.

Inflammatory states such as sepsis or endotoxemia lead to the expression of an inducible form of nitric oxide (NO) synthase (iNOS) in vascular tissues (25, 40). Production of NO by iNOS in various systemic vessels after LPS contributes to the hyporeactivity to vasoconstrictors in the whole animal (32) as well as in the isolated thoracic aorta (AO) (5, 12, 32) and femoral artery (35) rings. iNOS can produce up to a 1,000-fold greater concentration of NO than the constitutive isofoms, endothelial (e)NOS, and neuronal NOS, leading to vasodilatation and cytotoxicity (25).

Although it is generally accepted that iNOS upregulation in the systemic circulation is responsible for sepsis-induced hypotension, its role in the pulmonary vasculature’s development of hypertension is not well understood. LPS clearly increases lung iNOS expression (14, 43), and iNOS mRNA has been detected in the pulmonary artery (PA) after in vivo LPS (10). iNOS mRNA induction and increased nitrite production were also seen after in vitro stimulation with a mixture of cytokines and LPS in both PA endothelial (8) and smooth muscle cells (27). However, studies (3, 16) using immunolocalization techniques in LPS-induced acute lung injury do not report the presence of iNOS protein in the vascular smooth muscle of the PAs. Controversy also exists regarding the physiological role of iNOS-derived NO in the pulmonary circulation. The same group of investigators (9) who found evidence of iNOS mRNA induction after LPS also demonstrated increased PA pressures with selective iNOS inhibition and suggested a protective role for iNOS. Other investigators (23, 29) failed to confirm these findings; selective iNOS inhibition did not augment PA pressure in endotoxemia. In various experimental models of sepsis, we

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and others (7, 28, 38, 42) have found intact pulmonary arterial responses to multiple forms of vasoconstriction, suggesting the lack of iNOS-derived NO production in the PA. On the basis of these observations, we hypothesized that the differential response of specific vessels to injury or stress may be due to phenotypic heterogeneity between vascular cell populations. Specifically, we hypothesized that iNOS was responsible for endotoxin-induced vascular hypocontractility in AO but not PA vessels.

The purpose of our investigation was to determine the role of iNOS in the rat AO and PA after endotoxin administration. Vascular responses to α1-adrenergic stimulation were determined in the presence of nonselective NOS and selective iNOS inhibition. We then examined these vessels for the presence of iNOS protein using Western blot and immunohistochemical techniques. The results of this study demonstrate that endotoxin upregulates iNOS in AO but not PA vessels.

**MATERIALS AND METHODS**

Animal care and housing. All animals received humane care in compliance with the National Research Council's Guide for the Care and Use of Laboratory Animals. Male Sprague-Dawley rats weighing 250–300 g were quarantined in quiet, humidified, light-cycled rooms for 2–3 wk before use. Rats were allowed ad libitum access to food and water throughout quarantine.

Experimental protocol. Rats were administered normal saline (NS, 1 ml ip) or Salmonella typhimurium LPS (20 mg/kg in 1 ml ip NS). Rats were provided chow and water ad libitum during the 6-h period after the second injection. No rats died during the 6-h experimental time course. A previous experiment using the same dose of LPS resulted in a 15% mortality at 72 h (unpublished data). After 6 h, the AO and PAs were incubated with N°-monomethyl-L-arginine (l-NMMA, 100 µmol/l), aminoguanidine (AG, 100 µmol/l), or an equivalent volume (100 µmol/l) of NS for 30 min.

Isolated AO and PA ring preparation. Isolated arterial rings were harvested and prepared as previously described (7, 37). Rats were anesthetized with pentobarbital sodium (50 mg/kg ip) at 6 h. Median sternotomy was performed, and heparin sulfate (500 USP units) was injected into the right ventricular outflow tract. After the removal of the heart and lungs en bloc, the AO and the left and right PAs were excised. The aorta and the right and left main branch PAs were then cut into 3-mm-wide rings; two aorta and two PA rings were obtained from each rat. Care was taken during this process to avoid endothelial injury.

The aorta and PA rings were then placed on 11-ml gauge steel wires and suspended in individual 10-ml tissue chambers containing Earl's balanced salt solution (EBSS), a standard physiological buffer consisting of (in mM) 1.80 CaCl2, 0.83 MgSO4 (anhydrous), 5.36 KCl, 116.34 NaCl, 0.40 NaPO4 (dibasic), 5.50 d-glucose, and 19.04 NaHCO3. The tissue chambers were surrounded by water jackets and continually warmed (37°C). Ring tension was determined with the use of a force displacement transducer (Grass FT03, Grass Instruments, Quincy, MA) attached to each steel wire apparatus. Force displacement was recorded at 0.67 Hz using a MacLab Data Interface Module (ADI Instruments, Milford, MA) on a Macintosh Quadra 650 computer (Apple Computer, Cupertino, CA). Each tissue chamber had continual bubbling gas flow at 40 ml/min of 21% O2, 5% CO2, and 74% N2. This produced a PO2 of 100–110 mmHg, pH of 7.4.

AO and PA response to vasoconstriction. The optimal resting mechanical tension (passive load) was determined to be 750 mg for pulmonary rings and 1,000 mg for AO rings in prior experiments. Rings were suspended at 750 mg (PA) or 1,000 mg (AO) and allowed to reach a steady state for 1 h, during which time EBSS was changed at 30 min. At 30 min, an NOS inhibitor (l-NMMA or AG, 100 µmol/l) or an equivalent volume of NS (100 µmol/l) was added to the tissue bath. Cumulative concentration-response curves to phenylephrine (PE) were then generated over the concentration range of 10−9 to 10−3 M. For determination of the concentration-response curve, the ring was allowed to reach a steady state before advancing to the next higher concentration. The tension present in the ring in response to each dose of PE was expressed in milligrams. Four to six rats (8–12 rings) were studied in all groups.

Immunoblotting. After dissection of the AO and PA rings at 6 h from the saline and LPS-treated rats as described in the Experimental protocol, the samples were placed on ice in five volumes of a homogenization buffer containing (in mM) 25 Tris-HCl, 2 EGTA, and 1 PMSF, pH 7.4. The samples were homogenized for 30 s with a Tissumizer (Tekmar, Cincinnati, OH). The suspension was centrifuged at 3,000 g for 5 min at 4°C. The protein concentration of the supernatant was determined with the use of the Coomassie Plus protein assay (Pierce, Rockford, IL). Samples (20 µg of crude protein) were mixed with an equal volume of sample buffer (100 mM Tris-HCl, 2% SDS, 0.02% bromophenol blue, and 10% glycerol) and boiled for 5 min. Electrophoresis was performed on 4–20% linear gradient SDS polyacrylamide gels. Proteins were then electrophoretically transferred onto nitrocellulose membranes (Bio-Rad, Hercules, CA). Membranes were blocked for 1 h at room temperature with antibody buffer (PBS containing 0.1% Tween 20 and 5% nonfat dried milk) and then incubated with primary antibody (rabbit polyclonal anti-iNOS, Affinity Bioreagents, Golden, CO; 1:500 dilution with antibody buffer) for 18 h at 4°C. This antibody has been used previously to identify rat iNOS for both Western blot and immunohistochemistry (1). Membranes were washed three times in PBS containing 0.1% Tween 20 and then incubated with peroxidase-labeled goat anti-rabbit IgG (Jackson Laboratories, West Grove, PA; 1:1,000 dilution with antibody buffer) for 1 h at room temperature. Membranes were then washed three times, and antigen-antibody complexes were revealed by enhanced chemoluminescence.

Quantification of the immunoblot was performed with a computerized laser densitometer (Molecular Dynamics, Sunnyvale, CA). Density values are expressed relative to the saline control level of each experiment. All densities reported are means ± SE of three separate experiments.

Immunohistochemistry. After dissection of the AO and right and left PA rings, the tissue was embedded in a tissue-freezing medium. The tissue was then rapidly frozen in dry ice-cooled 2-methylbutane and stored at −70°C. Transverse 5-µm cryosections were prepared with a cryostat (IEC Minom Plus, Needham Heights, MA), collected on glass slides, and air dried for 60 min. All sections were fixed for 10 min in a 70% acetone-30% methanol mixture at −20°C. Sections were then blocked with 10% normal goat serum and incubated with primary antibody (rabbit polyclonal anti-iNOS, Affinity Bioreagents; 1:50 dilution with PBS containing 1% BSA) for 1 h. After three washes with PBS, sections were incubated for 45 min with Cy3 (red)-labeled goat anti-rabbit IgG (Jackson Laboratories; 1:50 dilution with PBS containing 1% BSA). After being washed with PBS, sections were counterstained with Oregon green-labeled wheat germ agglutinin (5 µg/ml for glycoprotein staining) and bis-benzimide (blue; 10 µg/ml...
for nuclear staining). Sections were then mounted with aqueous antifluorescin medium. To assess the specificity of the immunostaining, adjacent sections were incubated with nonimmune rabbit IgG (5 µg/ml in PBS containing 1% BSA) in replacement of the primary antibody and then processed identically. Microscopic observation and photography were performed with a Leica DMRXA microscope (Germany) equipped with digital confocal software by Intelligent Imaging Innovations (Denver, CO).

Reagents. Standard reagents as well as the Salmonella typhimurium LPS were obtained from Sigma Chemical (St. Louis, MO). Fresh solutions were prepared daily with either deionized water or NS as the diluent. The concentrations are expressed as final molar concentrations in the organ chambers.

Statistical analysis. Statistical analyses were performed with a MacIntosh Quadra computer and StatView software (Brain Power, Calabasas, CA). Data are presented as means ± SE of the number of rings studied at each point of data collection. Statistical evaluation utilized standard one-way ANOVA with post hoc Bonferroni Dunn t-test. A P value of <0.05 was accepted as statistically significant.

RESULTS

Effect of LPS on α1-adrenergic receptor stimulation. PA ring contraction in response to α1-adrenergic receptor stimulation PE was not impaired by in vivo LPS administration (Fig. 1C). The maximal tension developed in response to PE (at 10⁻⁵ M) was similar for both control (466 ± 29 mg) and LPS-treated (455 ± 27 mg) rats (P = 0.79). In contrast, AO ring contraction was markedly impaired by LPS (Fig. 1D). The maximal tension developed in response to PE (at 10⁻⁵ M) was 1,076 ± 3 mg in control vs. 412 ± 39 mg in LPS-treated rats (P < 0.05).

Effect of nonselective NOS inhibition. The effect of α1-adrenergic receptor stimulation at all doses studied (10⁻⁹ to 10⁻³ M) was potentiated by the nonselective NOS inhibitor L-NMMA in both PA and AO rings from control rats (P < 0.05 vs. control, Fig. 1, A and B). In PA rings from LPS-treated rats, L-NMMA similarly increased the effect of PE (maximal tension: LPS + L-NMMA 824 ± 75 mg vs. LPS 455 ± 27 mg, P < 0.05, Fig. 1C). In AO rings from LPS-treated rats, L-NMMA potentiated the response to PE at all doses studied (maximal tension: LPS + L-NMMA 1,512 ± 57 mg vs. LPS 412 ± 39 mg, P < 0.05 vs. LPS, Fig. 1D).

Effect of selective iNOS inhibition. AG did not affect the response to PE in PA and AO rings from control rats (P > 0.05, Fig. 2, A and B). In PA rings from LPS-treated rats, AG again did not change the response to PE (maximal tension: LPS 455 ± 27 mg vs. LPS + AG 422 ± 38 mg, P = 0.44, Fig. 2C). However, in AO rings from LPS-treated rats, AG restored the response to PE...
at the doses studied, \(10^{-9}\) to \(10^{-3}\) M (maximal tension at \(10^{-5}\) M: LPS + AG 1.135 ± 54 mg, \(P < 0.05\) vs. LPS and \(P > 0.05\) vs. control, Fig. 2D).

Immunoblotting for iNOS. The protein level of iNOS in the PA and AO was determined in control and ETX-treated rats (Fig. 3A). The 130 kDa of iNOS protein were undetectable in the PA and AO of control rats. Six hours after LPS, iNOS was found in the aorta but not in the main branch PAs. Densitometric analysis (means ± SE) of three separate experiments reveals an increase in iNOS in the AO from LPS-injected rats. (\(P < 0.05\) vs. all other groups, Fig. 3B).

Immunofluorescent analysis of iNOS. The distribution of iNOS in vascular tissue after ETX was examined by immunofluorescent localization. iNOS immunoreactivity was not detected in sections incubated with nonimmune rabbit IgG (data not shown). Immunostaining with rabbit polyclonal anti-iNOS antibody failed to detect significant levels of iNOS in both the control and LPS-treated PA (Fig. 4). In the AO, perinuclear iNOS is seen in the controls, but LPS resulted in the appearance of increased levels of iNOS throughout the vascular smooth muscle cells (Fig. 4).

**DISCUSSION**

We found that LPS leads to iNOS upregulation and vascular hyporeactivity in systemic (AO) but not in pulmonary (main branch PA) vessels. \(\alpha_1\)-Adrenergic receptor-mediated vasoconstriction was markedly impaired in the AO rings after in vivo LPS administration. Selective iNOS inhibition restored normal contractile responses to PE. This functional data was confirmed by the presence of iNOS protein on Western blot and immunohistochemical staining of the aorta of LPS-treated rats. However, LPS did not affect the PA's response to \(\alpha_1\)-adrenergic receptor-mediated vasoconstriction, and selective iNOS inhibition had no effect. Finally, iNOS protein was not detected in the PA by either Western blot or immunohistochemistry.

Previous observations (18) suggested that the effect of inflammatory states such as endotoxemia on vascular tone may differ between different vascular beds, resulting in a maldistribution of blood flow. Indeed, several investigators (20, 28, 42) found that vessels harvested from different vascular beds respond differently after LPS or live bacteria injection. We found that in vivo LPS had no effect on vasoconstriction to an \(\alpha_1\)-adrenergic agonist in isolated rat PA rings. However, AO rings from the same animal had a markedly diminished vasoconstrictive response. Other investigators made similar observations. Nelson et al. (28) found that LPS administration in the sheep resulted in depressed sensitivity to norepinephrine (NE) and KCl in the isolated superficial femoral artery but not in the tertiary branch PA. Similarly, Li and colleagues (20) found regional differences in the response of isolated vessels from the rabbit. LPS had no effect on vasoconstriction to NE or histamine in the renal artery but...
decreased contraction in the isolated ear artery. Suba et al. (42) also found that vasoconstriction to NE was depressed in the mesenteric circulation but not in the PA in a porcine sepsis model.

Our findings that LPS leads to an increase in iNOS causing a hypocontractile state in the AO ring confirm the work of other investigators (12, 28, 34–36). We found that nonselective NOS and selective iNOS inhibition both ameliorated the hypocontractile state after LPS. Furthermore, we found increased AO iNOS protein by Western blot analysis as well as immunohistochemistry. Julou-Schaeffer et al. (12) found that LPS caused a right shift of the dose response to NE in AO rings and a reduction of the maximal contraction by 43% and 54% with and without functional endothelium. They also found that the effects of LPS were potentiated by L-NMMA and blocked by nonselective NOS inhibition. Other investigators (15, 41) determined that LPS is able to induce the expression of iNOS in the rat aorta smooth muscle cells. Vasomotor dysfunction in the AO is not limited to hypocontractility. We (21, 22) previously found that LPS causes dysfunction of vasorelaxation to both cGMP- and cAMP-mediated agonists. Parker and Adams (30) found that LPS causes selective inhibition of endothelium-dependent relaxation in guinea pig AO rings.

LPS injection in the rat results in an acute lung injury characterized by lung neutrophil accumulation, edema, increased pulmonary vascular resistance, and dysfunction of vasorelaxation to cGMP-mediated agonists (4, 7, 37). Although early production of NO by eNOS after LPS appears to play a protective role in acute lung injury (11), the later phase of endotoxemia results in the induction of pulmonary iNOS (14, 43), with subsequent production of NO and its cytotoxic derivative peroxynitrite. Although the LPS-induced increase in pulmonary iNOS activity has been extensively studied, the enzyme’s role in the pulmonary circulation is not well understood. We found that decreased contraction in the isolated ear artery, Suba et al. (42) also found that vasoconstriction to NE was depressed in the mesenteric circulation but not in the PA in a porcine sepsis model.

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pulmonary vasoconstriction to the \( \alpha_1 \)-adrenergic agonist PE was not different from the control after LPS injection, and selective iNOS inhibition did not augment the contractile response, in contrast to our findings in the AO. Furthermore, we found that LPS did not induce iNOS in the main PA by either Western blot analysis or immunohistochemistry. Nonselective NOS inhibition caused an increase in baseline tension in the PA ring. This finding is likely due to inhibition of eNOS, confirming the importance of endothelial-derived NO in maintaining a low basal tone in the pulmonary circulation. It is interesting that this finding only occurred in the LPS-injected rats. Other compensatory factors may override this effect in the control PA ring, such as prostacyclin release. This observation was not found in the AO rings. The systemic basal tone is much higher in the AO ring compared with the PA, and eNOS may play a less important role.

Past investigations of PA iNOS had mixed results. In vitro studies (8, 27) suggested that rat PA smooth muscle and endothelium iNOS mRNA are induced when cultured cells are stimulated by a combination of cytokines and LPS. Furthermore, Griffiths et al. (10) reported the presence of iNOS mRNA in the PA after in vivo LPS, which was associated with hypococontractility to KCl and PE. This same group of investigators (9) found that in isolated rat lungs, LPS increased baseline PA pressures; however, vasoconstriction to hypoxia and prostaglandin F\(_2\alpha\) were not different from the sham-treated rats. Despite increased baseline PA pressures, AG further increased PA pressure in the LPS-treated group. The above findings contrast with those of the current study and previous work by Nelson et al. (28) and Suba et al. (42). Nelson et al. (28) found no effect of LPS on NE and KCl in the tertiary branch PA. Suba et al. (42) found that vasoconstriction to NE was normal in the PA in a porcine sepsis model. Studies of in vivo selective iNOS inhibition on pulmonary hemodynamics in LPS-induced acute lung injury also conflict with the findings of Griffiths et al. (9 and 10) in the isolated lung. In two different LPS models of acute lung injury, in vivo selective iNOS inhibition did not augment mean PA pressure, suggesting a lack of iNOS expression in pulmonary vessels (23, 29). In contrast to the current study, the above investigations did not include positive controls, demonstrating that the dose of LPS used caused an increase in iNOS in other tissues (i.e., systemic vessels).

Although our data suggest that LPS does not increase iNOS in pulmonary vessels, iNOS is found in the lung in inflammatory states. Carraway et al. (3) found that iNOS was not detectable in normal rat lungs by either Western blot or immunohistochemistry. However, after cecal ligation and puncture, iNOS was strongly expressed in alveolar and bronchial epithelium, capillary endothelium, macrophages, and Clara cells (3). In LPS-induced acute lung injury, immunohistochemical localization of iNOS revealed its presence in the respiratory epithelium, capillary endothelium, and alveolar macrophage (16). Recently, Fujii et al. (6) implicated pulmonary macrophage activation in sepsis as the major determinant of lung NO production. Blockade of LPS-induced macrophage activation eliminated the increase in lung iNOS activity and protein expression and attenuated the increase in exhaled NO (6). Although iNOS inhibition appears to neither benefit nor harm PA vasomotor function in endotoxemia, other important aspects of acute lung injury are significantly attenuated or even abrogated. Selective iNOS inhibition with either AG or S-methylisothiourea in a rat model of LPS-induced acute lung injury prevented the increase in lung iNOS activity and reduced lung vascular leak (2). Mice deficient in the iNOS gene are more resistant to LPS-induced acute lung injury than corresponding wild-type mice, as evidenced by the lack of changes in lactate dehydrogenase activity, lung wet/dry ratio, and pulmonary nitrotyrosine staining after LPS (17).

The vasomotor effects of iNOS-produced NO may vary, depending on the local NO tissue concentration, the specific mechanism producing vasoconstriction, and the type of vessel (conductance or resistance) being studied (39). Although the LPS-induced increase in vascular iNOS and subsequent marked hyporeactivity has been well characterized in large elastic arteries such as the rat aorta, other investigators (24, 35) failed to find significant vascular dysfunction in smaller resistance arteries, despite a measured increase in iNOS activity. Kleschyov et al. (13) recently implicated the adventitia as an important source of NO, and the relatively greater amounts of adventitial cells in the large conductance arteries versus the smaller resistance vessels may explain these divergent findings. In the pulmonary circulation, the contractile and pharmacological responses of large and small arteries are also not identical; the vascular reactivity varies depending on the agonist employed (19). iNOS may be present in smaller PAs in sepsis, but other studies (23, 29) failed to find a pathophysiological role for the enzyme in these resistance vessels.

In summary, we examined the effects of LPS on vascular reactivity and iNOS expression in systemic and pulmonary conductance vessels. As previous studies have found, LPS increases iNOS expression in the AO and impairs \( \alpha_1 \)-adrenergic receptor-mediated vasoconstriction. However, LPS does not induce iNOS expression in the PA, and selective iNOS inhibition does not affect the responses to vasoconstriction in the PA rings from either control or LPS-treated rats. From these data, we conclude that there is differential iNOS expression in systemic and pulmonary vessels in response to LPS. This difference in regional iNOS expression contributes to the phenomenon of sepsis/endotoxemia-induced systemic hypotension and pulmonary hypertension.

Perspectives

Investigators have long recognized that endotoxemia is characterized by maldistribution of blood flow that is different between different vascular beds (18). The most obvious changes are pulmonary hypertension coupled with systemic hypotension. This dichotomous
response has never been completely understood. Early after the discovery of NO, investigators (5, 12, 32) recognized that iNOS induction leads to vascular hypotreactility in AO or femoral artery rings. However, the same findings were not observed in the pulmonary circulation (7, 28, 38, 42). The current study demonstrates that these observations are, in part, due to phenotypic differences in smooth muscle between the AO and PA in the rat. We are not the first to make such an observation. Gieger et al. (8) found that rat aortic endothelial cells had substantial induction of iNOS in response to interferon-γ (IFN-γ), tumor necrosis factor-α, and LPS. In contrast, rat lung microvascular endothelial cells had very little induction of iNOS (8). Similarly, Murphy et al. (26) recently found a marked difference in NO generation by lung and dermal endothelial cells in response to IFN-γ and LPS. The current study adds to this growing body of literature suggesting that cell populations from different organs have significant heterogeneity in iNOS induction that may lead to a different response to or susceptibility to injury.

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REFERENCES


