Anesthesiology
1999; 91:1724–32

Selective iNOS Inhibition Attenuates Acetylcholine- and Bradykinin-induced Vasoconstriction in Lipopolysaccharide-exposed Rat Lungs

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Background: **Nonselective nitric oxide synthase (NOS) inhibition has detrimental effects in sepsis because of inhibition of the physiologically important endothelial NOS (eNOS). The authors hypothesized that selective inducible NOS (iNOS) inhibition would maintain eNOS vasodilation but prevent acetylcholine- and bradykinin-mediated vasoconstriction caused by lipopolysaccharide-induced endothelial dysfunction.**

Methods: **Rats were administered intraperitoneal lipopolysaccharide (15 mg/kg) with and without the selective iNOS inhibitors L-*N*6-(1-iminoethyl)-lysine (L-NIL, 3 mg/kg), dexamethasone (1 mg/kg), or the nonselective NOS inhibitor N^{ω}-nitro-L-arginine methylester (L-NAME, 5 mg/kg). Six hours later, the lungs were isolated and pulmonary vasoreactivity was assessed with hypoxic vasoconstrictions (3% O_2), acetylcholine (1 μg), Biochemical Engineering, and bradykinin (3 μg). In additional lipopolysaccharide experiments, L-NIL (10 μM) or 4-Diphenylacetoxy-*N*-methylpiperidine methiodide (4-DAMP, 100 μM), a selective muscarinic M_3 antagonist, was added into the perfusate.**

Results: **Exhaled nitric oxide was higher in the lipopolysaccharide group (37.7 ± 17.8 ppb) compared with the control group (0.4 ± 0.7 ppb). L-NIL and dexamethasone decreased exhaled nitric oxide in lipopolysaccharide rats by 83 and 79%, respectively, whereas L-NAME had no effect. In control lungs, L-NAME significantly decreased acetylcholine- and bradykinin-induced vasodilation by 75% and increased hypoxic vasoconstrictions, whereas L-NIL and dexamethasone had no effect. In lipopolysaccharide lungs, acetylcholine and bradykinin both transiently increased the pulmonary artery pressure by 8.4 ± 2.0 mmHg and 35.3 ± 11.7 mmHg, respectively, immediately after vasodilation. L-NIL and dexamethasone both attenuated this vasoconstriction by 70%, whereas L-NAME did not. The acetylcholine vasoconstriction was dose-dependent (0.01–1.0 μg), unaffected by L-NIL added to the perfusate, and abolished by 4-DAMP.**

Conclusions: **In isolated perfused lungs, acetylcholine and bradykinin caused vasoconstriction in lipopolysaccharide-treated rats. This vasoconstriction was attenuated by administration of the iNOS inhibitor L-NIL but not with L-NAME. Furthermore, L-NIL administered with lipopolysaccharide preserved endothelium nitric oxide–dependent vasodilation, whereas L-NAME did not. (Key words: Endothelium; L-*N*-(1-iminoethyl)-lysine; nitric oxide; pulmonary circulation; sepsis.)**

ORGAN system dysfunction caused by altered perfusion, systemic hypotension, and hyporesponsiveness to catecholamines are common disorders associated with sepsis. Lungs are very susceptible to changes seen in endotoxemia. Acute respiratory distress syndrome, one of the most common consequences of sepsis,[1] is a significant cause of morbidity and mortality in patients.[1] Many cofactors and mediators are involved in sepsis. Perhaps most importantly is the free radical nitric oxide (NO), which is synthesized from the guanidino group of L-arginine by a family of enzymes called nitric oxide synthases (NOS). Three isoforms have been identified. The cerebral NOS is present in the central and peripheral nervous systems.[1] NO produced by endothelial NOS (eNOS) is involved in regulation of blood pressure, blood flow distribution, and inhibition of platelet adhesion to the endothelium.[1] Inducible NOS (iNOS) can be increased by multiple proinflammatory agents and is found in a variety of mammalian cells, including macrophages,

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Received from the Department of Anesthesiology, University of Virginia, Health Sciences Center, Charlottesville, Virginia. Submitted for publication October 15, 1998. Accepted for publication June 10, 1999. Dr. Fischer was supported by Innovative Medizinische Forschung, Münster, Germany (Fi-6-2-98-01).

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vascular smooth muscle cells, and respiratory epithelial cells.[1,2] iNOS has bactericidal properties against invasive agents, but also its prolonged overproduction is partially responsible for circulatory collapse,[3] myocardial depression,[4] and microvascular injury.[5]

Inducible NOS has been shown to have high activity in rat lungs 3 to 4 h after application of lipopolysaccharide.[6] Overproduction of NO associated with lipopolysaccharide may lead to endothelial dysfunction in the pulmonary circulation.[7] Attempts to decrease iNOS with nonselective NOS inhibition causes detrimental effects in septic models. Robertson *et al.*[8] showed that induced pulmonary hypertension was potentiated by infusion of N^G-nitro-L-arginine methylester (L-NAME). L-NAME has significantly decreased survival times in septic mice[9] and rats.[10] Additionally, nonselective NOS inhibition has detrimental effects in the liver[11] and may decrease mucosal blood flow through the intestines[12] and cardiac output because of an increase in vascular resistance.[13] These results occur because eNOS has physiologically important functions in maintaining organ blood flow, regulation of adhesion and activation of blood cells, and microvascular protection,[7] all of which are decreased by L-NAME. L-*N*6-(1-iminoethyl)-lysine (L-NIL) is a new L-arginine analogue that is a potent and relatively selective inhibitor of iNOS.[14] Stenger *et al.*[15] showed that L-NIL has a 30-fold higher selectivity for the inducible compared with the endothelial isoform of the NOS enzyme. Schwartz *et al.*[16] demonstrated in rats that L-NIL decreased urinary nitrite–nitrate excretion and prevented the hypotension and the decrease in glomerular filtration rate associated with lipopolysaccharide administration. Dexamethasone, which is an inhibitor of iNOS at the transcriptional level, also has been shown to inhibit iNOS in aortic rings during sepsis.[17]

We hypothesized that selective inhibition of iNOS would maintain eNOS-mediated or endothelium-dependent vasodilation and prevent acetylcholine- and bradykinin-mediated vasoconstriction, which occurs with lipopolysaccharide-induced endothelial dysfunction. In this study, we administered rats intraperitoneal lipopolysaccharide from *Salmonella typhimurium* (15 mg/kg) with and without the concurrent administration of L-NIL and dexamethasone as selective iNOS-inhibitors and L-NAME as a nonselective NOS inhibitor. Six hours later, the lungs were isolated and vasoreactivity was determined with hypoxic challenges (3% O_2) and with endothelium-dependent (acetylcholine, bradykinin) and endothelium-independent (sodium nitroprusside [SNP]) vasoactive agents.

Materials and Methods

Experimental Groups

This study was approved by the animal research committee at the University of Virginia. Rats were allowed food and water *ad libitum.* The following groups (n = 4–6) of male rats (250–350 g, Harlan Sprague-Dawley) were studied: Groups 1–3 were control groups (no lipopolysaccharide) given saline, L-NIL (3 mg/kg), or L-NAME (5 mg/kg). Groups 4–7 received lipopolysaccharide (15 mg/kg) and saline, L-NIL (3 mg/kg), dexamethasone (1 mg/kg), or L-NAME (5mg/kg). Lipopolysaccharide and all drugs were administered intraperitoneally 6 h before evaluation using an isolated lung preparation.

Isolated Rat Lung Preparation

Rats were anesthetized with α-chloralose (50 mg/kg) and urethane (650 mg/kg) intraperitoneally. A 17-gauge cannula was inserted into the trachea *via* a tracheostomy, and the lungs were ventilated with warmed (35°C) and humidified 21% O_2, and 5% CO_2, balanced nitrogen, using a rodent ventilator (tidal volume = 1 ml/100 g, frequency = 60 breaths/min). End-expiratory pressure was set at 1 mmHg. After sternotomy, sections of the right and left anterior chest wall were excised to expose the heart and lungs. The rat was heparinized (100 U), then partially exsanguinated by needle aspiration (6–8 ml). A 13-gauge steel cannula, connected to the perfusion system, was inserted through the pulmonic valve into the main pulmonary artery *via* an incision in the right ventricle. The cannula was secured by a suture tied around the pulmonary artery and aorta that prevented systemic blood flow. A 3.5-mm-OD cannula was inserted through the apex of the left ventricle and secured with umbilical tape around the ventricles.

Perfusate consisted of the rat's own blood (added to the perfusate after lung isolation) diluted with physiologic salt solution to a hematocrit of 9–12%. Indomethacin (30 μg/ml perfusate) was added to block prostaglandin synthesis. Perfusate drained from the left ventricle to a glass reservoir and was heated to 38°C by a circumferential water jacket. Perfusate was returned to the pulmonary artery at constant flow (16 ml/min) using a peristaltic pump (Masterflex, Barrington, IL). The isolated lung preparation remained in the thoracic cavity, which lay supine on a heated plate. A warmed and humidified chamber was placed over the thoracic cavity to maintain thoracic temperature at 37°C. Reservoir pH was continuously monitored (Cole-Parmer Inst., Chi-

cago, IL) and maintained at 7.35–7.45 by addition of HCl or NaOH as necessary.

Pulmonary artery pressure (P_a) was monitored continuously using a pressure transducer (Abbott Laboratories, North Chicago, IL). Mean pulmonary venous pressure (P_v) was set at 2 mmHg by adjusting the height of the reservoir and was held constant. Normoxic (21% O_2) and hypoxic (3% O_2) gas mixtures were administered through individual flowmeters. The inspired oxygen concentration was monitored (Fraser-Harlake, Orchard Park, NY) near the tracheal tube.

Experimental Protocol

After tracheostomy, the rats underwent ventilation and the exhaled air was collected in a plastic bag for 5 min. The exhaled NO concentration was measured using a chemiluminescence detector (NOA 280; Sievers, Boulder, CO). The lungs were then isolated and the perfusion rate was stepped up to 16 ml/min. After a 10-min stabilization period, hypoxic responses were elicited. The lungs were ventilated with the hypoxic mixture (3% O_2, 5% CO_2, 92% N_2) for 10 min and then again with the normoxic mixture (21% O_2, 5% CO_2, 74% N_2) for an equal period. The sequence was repeated one time. Next, the thromboxane analogue U-46619 (9-11-dideoxy-11α-9α-epoxymethano-prostaglandin $F^2\alpha$) was added to the reservoir to increase the P_a to 25–30 mmHg. After stabilization, endothelium-dependent responses were evaluated, with 1.0 μg acetylcholine injected into the inflow of the circuit. Five minutes after the P_a returned to baseline, the sequence was repeated with bradykinin (3 μg) and the endothelium-independent vasodilator SNP (25 μg). All drugs were given in concentrations used before in this model.[18]

Additional experiments (n = 6, each group) were performed in lipopolysaccharide-exposed rats to determine whether the acetylcholine-induced vasoconstriction and vasodilation was dose-dependent, whether acutely decreasing iNOS would prevent acetylcholine-induced vasoconstriction, and whether the vasoconstriction was caused by stimulation of the muscarinic M_3 receptor. After isolation of the lung, lipopolysaccharide-exposed rats were studied with and without L-NIL (10 μM) or muscarinic antagonist 4-Diphenylacetoxy-*N*-methylpiperidine methiodide (4-DAMP, 100 μM) added to the perfusate and using acetylcholine in three different concentrations (0.01 μg, 0.1 μg, and 1.0 μg). Control lungs were administered the three concentrations of acetylcholine for comparison to lipopolysaccharide-exposed lungs.

Solutions

Sodium nitroprusside, L-NAME (both Sigma Chemicals, St. Louis, MO) and 4-DAMP (Research Biochemicals Incorporated, Natick, MA) were prepared by dilution in normal saline and stored at 4°C. Acetylcholine and bradykinin (Sigma Chemicals, St. Louis, MO) were prepared by dilution in normal saline and stored at −20°C. U 46619 (Sigma Chemicals) was dissolved in 95% ethanol and stored at −20°C. L-NIL (Alexis Corporation, San Diego, CA) was dissolved in normal saline and stored at −20°C. Pilot studies showed that each of these vehicles had no effect on P_a.

Calculations and Statistical Analysis

Vasoconstriction from hypoxic vasoconstrictions, acetylcholine, bradykinin, and U 46619 were expressed as the peak P_a − baseline P_a. Vasodilation from acetylcholine, bradykinin, and SNP were expressed as a percentage (change in P_a divided by the P_a after U 46619 administration). Data are presented as the mean ± SD. SigmaStat 2.0 (SPSS, Inc., Chicago, IL) was used for statistical analysis. The various treatments were compared with one-way analysis of variance. When the data were not normally distributed, data were compared with Kruskal-Wallis test by analysis of variance. $P < 0.05$ was considered significantly different.

Results

General

All rats survived 6 h of lipopolysaccharide administration and underwent successful lung isolation. The baseline P_a in the isolated lung preparation was not significantly different between the lipopolysaccharide groups (12.5 ± 1.1 mmHg) and the control groups (11.7 ± 1.5 mmHg). Treatment did not alter the P_a except in the lipopolysaccharide + dexamethasone group (15.7 ± 2.2 mmHg), in which it was significantly increased. The amount of U 46619 needed for increasing the P_a between 25 and 30 mmHg was not different (mean = 0.7 ± 0.2 μg) between the groups with the exception of the lipopolysaccharide + dexamethasone group (0.4 ± 0.04 μg).

Exhaled Nitric Oxide

In control rats exhaled NO (exNO) levels were very low and not altered by either L-NIL or L-NAME (fig. 1). Lipopolysaccharide significantly increased exNO. L-NIL and dexamethasone significantly decreased exNO in li-

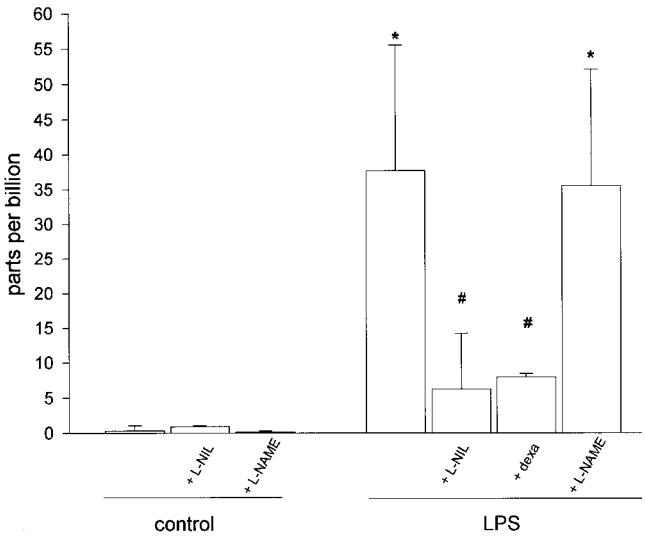

Fig. 1. Exhaled nitric oxide (parts per billion) measured 6 h after rats were administered lipopolysaccharide or saline (control). Control and lipopolysaccharide-exposed rats were concurrently administered saline, L-NIL, L-NAME, or dexamethasone (dexa). *Denotes significant ($P < 0.05$) increase from untreated control. #Denotes significant ($P < 0.05$) decrease from untreated lipopolysaccharide. Data are expressed as the mean ± SD.

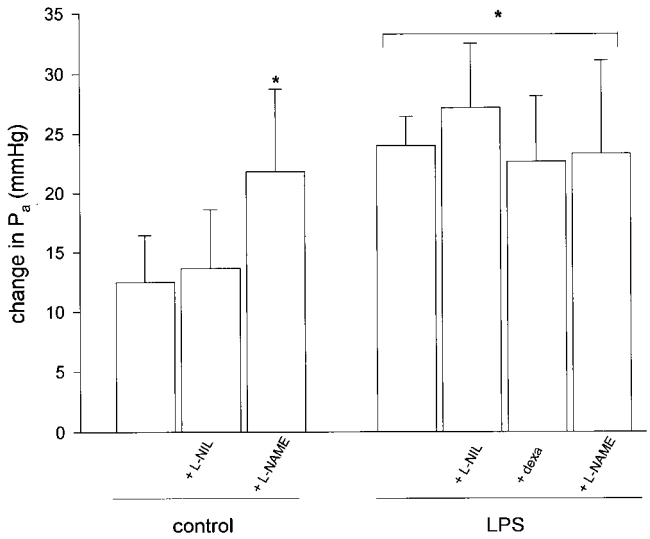

Fig. 2. Hypoxic pulmonary vasoconstriction (change in P_a, mmHg) measured in isolated rat lungs 6 h after the rats were administered lipopolysaccharide or saline (control). Control and lipopolysaccharide-exposed rats were concurrently administered saline, L-NIL, L-NAME, or dexamethasone (dexa). *Denotes significant ($P < 0.05$) increase from untreated control. Data are expressed as the mean ± SD.

popolysaccharide-exposed rats, whereas L-NAME did not.

Hypoxic Pulmonary Vasoconstriction

L-NIL did not alter the control hypoxic pulmonary vasoconstriction (HPV) response, whereas L-NAME caused a significant increase in the HPV response (fig. 2). Lipopolysaccharide significantly increased the HPV response compared with controls, but neither L-NIL, dexamethasone, or L-NAME altered the HPV response compared with lipopolysaccharide alone.

Responses to Acetylcholine and Bradykinin

Acetylcholine and bradykinin vasodilation was significantly decreased by L-NAME in control and lipopolysaccharide lungs. In contrast, L-NIL had no effect on vasodilation (fig. 3). Dexamethasone decreased vasodilation to bradykinin in lipopolysaccharide-exposed rats, but otherwise had no effect. Lipopolysaccharide administration did not alter acetylcholine or bradykinin vasodilation.

In control lungs, acetylcholine and bradykinin vasodilation was followed by a small vasoconstriction that was significantly increased by L-NAME but not L-NIL. In contrast, in lipopolysaccharide-exposed lungs, acetylcholine and bradykinin vasodilation was followed by a large

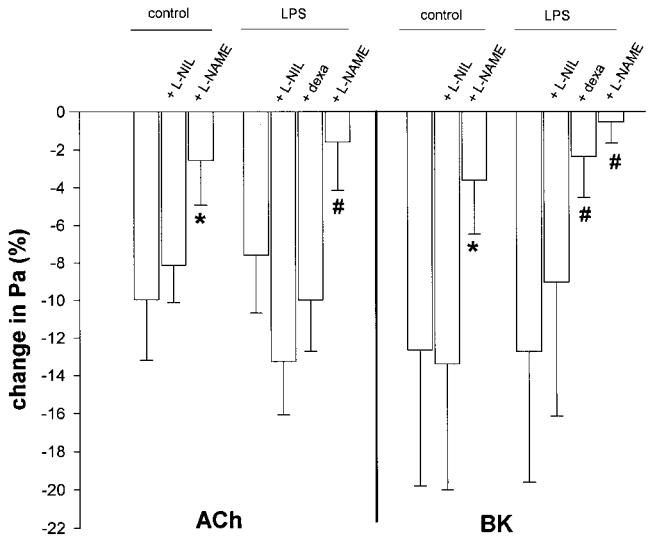

Fig. 3. Vasodilation (change in P_a, %) from acetylcholine (1 μg) and bradykinin (3 μg) measured in isolated rat lungs 6 h after the rats were administered lipopolysaccharide or saline (control). Control and lipopolysaccharide-exposed rats were concurrently administered saline, L-NIL, L-NAME, or dexamethasone (dexa). *Denotes significant ($P < 0.05$) decrease from untreated control. #Denotes significant ($P < 0.05$) decrease from untreated lipopolysaccharide. Data are expressed as the mean ± SD.

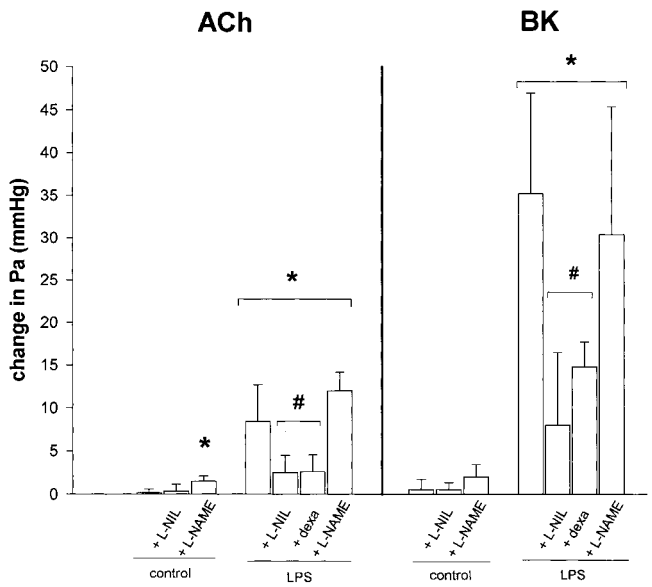

Fig. 4. Vasoconstriction (change in P_a, mmHg) from acetylcholine (1 μg) and bradykinin (3 μg) measured in isolated rat lungs 6 h after the rats were administered lipopolysaccharide or saline (control). Control and lipopolysaccharide-exposed rats were concurrently administered saline, L-NIL, L-NAME, or dexamethasone (dexa). *Denotes significant ($P < 0.05$) increase from untreated control. #Denotes significant ($p < 0.05$) decrease from untreated lipopolysaccharide. Data are expressed as the mean ± SD.

vasoconstriction (fig. 4, fig. 5). All lipopolysaccharide groups showed a significantly higher acetylcholine- and bradykinin-induced vasoconstriction compared with control lungs. L-NIL and dexamethasone, but not L-NAME, attenuated the increase in P_a induced by acetylcholine and bradykinin in the lipopolysaccharide groups (fig. 4).

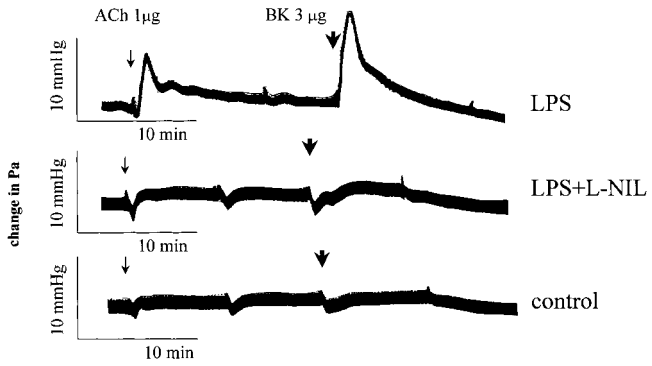

Fig. 5. Example of vasoconstriction (change in P_a, mmHg) from acetylcholine and bradykinin measured in an isolated rat lung 6 h after the rats were administered lipopolysaccharide, lipopolysaccharide plus concurrent administration of L-*N*6-(1-iminoethyl)-lysine (lipopolysaccharide + L-NIL), or saline (control).

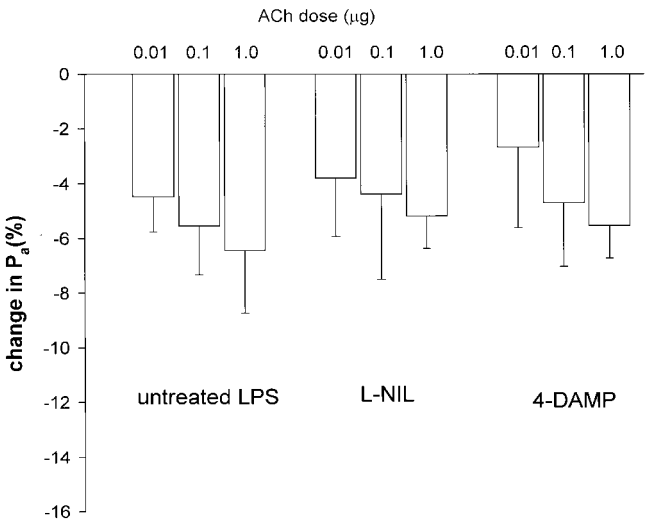

Fig. 6. Vasodilation (change in P_a, %) from acetylcholine (0.01 μg, 0.1 μg, and 1.0 μg) measured in isolated rat lungs 6 h after the rats were administered lipopolysaccharide. Saline, L-NIL (10 μM), or 4-DAMP (100 μM) was added to the perfusate. There were no significant differences. Data are expressed as the mean ± SD.

Responses to Sodium Nitroprusside

Sodium nitroprusside–induced vasodilation was not significantly altered by lipopolysaccharide (25.5 ± 2.6%) compared with control (24.2 ± 7.8%) responses. L-NIL, L-NAME, and dexamethasone did not alter the SNP vasodilation in control or lipopolysaccharide-exposed lungs.

Responses to L-NIL (10μM) and 4-DAMP (100 μM) in the Perfusate

There was a trend toward a dose-dependent vasodilation with acetylcholine in the lipopolysaccharide-exposed lungs; however, there was no significant difference among the three doses (0.01, 0.1, and 1.0 μg) (fig. 6). The vasodilation was not different compared with controls (6.7 ± 1.4%, 8.2 ± 2.7%, and 8.0 ± 3.8%) for the three acetylcholine doses. L-NIL or 4-DAMP added to the perfusate of the lipopolysaccharide-exposed lungs did not alter the acetylcholine vasodilation. Acetylcholine-induced vasoconstriction was significantly greater at 1.0 μg compared with either 0.01 or 0.1 μg in the lipopolysaccharide-exposed lungs. This vasoconstriction was not altered by L-NIL added to the perfusate but was nearly abolished by 4-DAMP (fig. 7). Exhaled NO was decreased by 86% when L-NIL was added to the perfusate.

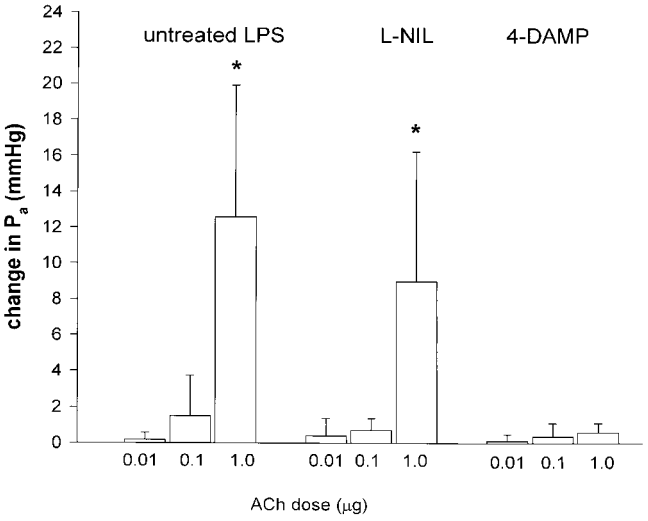

Fig. 7. Vasoconstriction (change in P_a, mmHg) from acetylcholine (0.01 μg, 0.1 μg, and 1.0 μg) measured in isolated rat lungs 6 h after the rats were administered lipopolysaccharide. Saline, L-NIL (10 μM), or 4-DAMP (100 μM) was added to the perfusate. *Denotes significant ($P < 0.05$) difference from all 4-DAMP and significant ($P < 0.05$) increase from acetylcholine 0.01 μg and 0.1 μg within each of group. Data are expressed as the mean ± SD.

Discussion

We evaluated endothelium-dependent and -independent vasoreactivity in isolated rat lungs to determine whether selective iNOS inhibition could maintain eNOS-mediated vasodilation but prevent acetylcholine- and bradykinin-induced vasoconstriction after lipopolysaccharide administration. The selective iNOS inhibitor L-NIL did not alter endothelium-dependent vasodilation or HPV in control or lipopolysaccharide-exposed lungs, which suggests that eNOS was not functionally altered. In contrast, L-NAME decreased endothelium-dependent vasodilation in control and lipopolysaccharide-exposed lungs and increased HPV in control lungs. In this model of sepsis, acetylcholine and bradykinin caused vasoconstriction, which is indicative of endothelial dysfunction. L-NIL and dexamethasone, but not L-NAME, significantly attenuated the acetylcholine- and bradykinin-induced vasoconstriction.

Exhaled NO was significantly increased by lipopolysaccharide administration. Concurrent administration of L-NIL or dexamethasone significantly attenuated the increase in exNO. This indicates that the elevated levels of exNO are secondary to increased iNOS. This is not surprising because the source of exhaled NO is mainly from alveolar macrophages and bronchial epithelium.[1,8] L-NAME did not alter the exNO level in lipopolysaccharide-exposed rats; however, it is possible that a higher dose may have attenuated the increase. Aaron *et al.*[19] demonstrated that 25 mg · kg^{-1} · h^{-1} intravenous L-NAME, which is 20-fold higher than our dose, significantly decreased exNO in rats. It is likely that this higher concentration of L-NAME blocks iNOS and eNOS.

L-NAME administered in a dose of 5 mg/kg intraperitoneally decreased acetylcholine and bradykinin vasodilation in control and lipopolysaccharide-exposed lungs and increased acetylcholine- and bradykinin-induced vasoconstriction in control lungs. This is consistent with other studies that have shown that L-NAME decreases systemic and pulmonary endothelium-dependent vasodilation as a result of eNOS inhibition.[20] In contrast, administration of L-NIL did not decrease acetylcholine- and bradykinin-induced vasodilation or increase vasoconstriction in control or lipopolysaccharide-exposed lungs; however, L-NIL attenuated acetylcholine- and bradykinin-induced vasoconstriction. Furthermore, L-NIL added directly to the perfusate in a concentration that decreased exNO did not alter endothelium-dependent vasodilation in lipopolysaccharide-exposed lungs. This result is important because it suggests that L-NIL maintains endothelium-dependent function, which requires eNOS. Maintaining eNOS has important physiologic implications in the regulation of blood pressure and blood flow distribution.[1] It is possible that selective iNOS inhibition may prevent additional organ damage in sepsis that occurs secondary to L-NAME–induced vasoconstriction.[11,12]

Hypoxic pulmonary vasoconstriction was significantly increased by L-NAME. This is consistent with previous studies that indicate that inhibition of the NO–cyclic guanosine monophosphate pathway increases HPV.[18,21] The observation that L-NIL did not alter HPV further suggests that L-NIL does not alter eNOS in the dose administered. The HPV was significantly increased by lipopolysaccharide, but not subsequently altered by L-NIL or L-NAME. Similarly, in pulmonary vascular rings from rats, Zelenkov *et al.*[22] showed that hypoxic constriction responses with 0% O_2 were enhanced after endotoxin treatment. It is surprising that the elevated NO from iNOS does not decrease HPV by direct inhibition of vascular smooth muscle contraction. However, it is possible that elevated NO is overcome by vasoconstrictive factors known to be released in sepsis, such as endothelin-1, leukotrienes, thromboxane A_2, and prostaglandins.[8] It is also interesting that inhibition of iNOS and eNOS did not increase HPV in lipopolysaccharide-exposed lungs; however, again, other vasoconstrictive factors may play a more important role during this con-

dition. Although indomethacin was added to the perfusate to eliminate the influence of arachidonic acid products, it is possible that their effect in the 6-h period before lung isolation contributes to the pulmonary vasoconstriction.

Endothelium-dependent vasodilation with acetylcholine and bradykinin did not appear to be affected by lipopolysaccharide. This is surprising because we would have expected lipopolysaccharide-induced endothelial dysfunction to decrease eNOS[23,24] and, therefore, endothelium-dependent vasodilation. Acetylcholine and bradykinin both bind with receptors on endothelial cells to release NO within the endothelial cells and stimulate the NO–cyclic guanosine monophosphate pathway.[1] Lipopolysaccharide may have also been expected to decrease endothelium-dependent vasodilation because the large increase in iNOS theoretically may result in decreased eNOS by feedback inhibition.[25,26] Vasodilation induced by SNP, which stimulates guanylate cyclase to cause vasodilation, was also not decreased, suggesting that the distal portion of the NO–cyclic guanosine monophosphate pathway is not altered. In contrast to our results, the studies of Zhou *et al.*[23] and Fullerton *et al.*[24] showed decreased eNOS and impaired function of endothelium-dependent and -independent vasodilation in septic rats. However, these investigators used thoracic aorta and pulmonary artery rings rather than isolated lungs. It is possible that the role of NO or the effect of lipopolysaccharide is different in larger vessels than in smaller resistance vessels evaluated in our study. It is also possible that the relatively low responses to endothelium-dependent vasodilators in our model were not sensitive enough to detect small changes in eNOS vasodilation. Although the level of endothelial dysfunction caused by 6 h of lipopolysaccharide administration did not appear to decrease endothelium-dependent vasodilation in our model, the pulmonary vasoconstriction caused by acetylcholine and bradykinin was increased dramatically.

Vasoconstriction caused by acetylcholine and bradykinin appears to be related to a receptor-mediated mechanism that is increased after lipopolysaccharide administration. In our lipopolysaccharide experiments, antagonism of muscarinic M_3 receptors nearly abolished the acetylcholine-induced vasoconstriction. Subtypes of acetylcholine receptors are known to be responsible for vasoconstriction. Organ- and animal-dependent M_1 or M_3 receptors are believed to be the cause of vascular smooth muscle contraction.[27–29] Recently, Hoover and Neely[28] showed that M_3 receptors mediate acetylcholine-induced vasoconstriction in rat coronary arteries. In simian coronary arteries, M_3 receptors were found at the vascular smooth muscle layer and caused constriction when activated.[29] Similarly, bradykinin receptor subtypes (bradykinin-1 or bradykinin-2) are responsible for vasodilation and vasoconstriction both. In endothelial denuded veins, Marsault *et al.*[30] and Hecker *et al.*[31] demonstrated vasoconstriction that was exclusively mediated by activation of the bradykinin-2 receptor subtype. We observed the same pattern of vasodilation and vasoconstriction for bradykinin as with acetylcholine after lipopolysaccharide.

Increased vasoconstriction caused by an acetylcholine receptor, probably at the vascular smooth muscle level, suggests endothelial dysfunction. It is likely that endothelial dysfunction allows for greater access of acetylcholine and bradykinin to the vascular smooth muscle,[30,31] and, hence, greater smooth muscle receptor–mediated responses. Meyrick *et al.*[32] showed lipopolysaccharide from *Escherichia coli* has direct toxic effects on the endothelium manifested by cell detachment, prostacyclin production, and cell lysis. Also, many of the vasoactive products induced in sepsis, such as tumor necrosis factor α, PAF, leukotrienes, and thromboxane A_2 can increase the endothelial permeability and integrity.[33] Therefore, it is likely that decreased functional endothelial integrity is the cause of the large vasoconstriction observed after acetylcholine and bradykinin administration. However, it is interesting that this endothelial dysfunction, which resulted in vasoconstriction, did not decrease endothelium-dependent vasodilation. This suggests that, in this particular lipopolysaccharide model, cellular NO is maintained but cellular functional integrity is altered.

Pulmonary vasoconstriction caused by acetylcholine and bradykinin was attenuated significantly by concurrent administration of either L-NIL or dexamethasone, but not L-NAME. This indicates that NO produced by iNOS is at least partially responsible for the vasoconstriction. It is possible that NO in large amounts damages the endothelium and alters receptor-mediated activity because of direct cytotoxic effects. NO and its nitrosative products damage cellular constituents and interfere with DNA synthesis and the structural integrity of the cell.[34,35] It is also possible that NO alters the cyclooxygenase pathways[36,37] that are involved in vasoreactivity. NO is known to participate in modulation of the enzyme alkaline phosphatase *via* activation of the cyclooxygenase pathway.[37] The observation that L-NIL added to the perfusate did not alter the vasoconstriction suggests that it is not the presence of large amounts of NO itself, but rather the long-term toxic

effects of NO on the endothelium that are responsible for the acetylcholine- and bradykinin-induced vasoconstriction. The observation that L-NAME did not attenuate the acetylcholine- and bradykinin-induced vasoconstriction in lipopolysaccharide-exposed lungs suggests that iNOS was not decreased despite the decrease in endothelium-dependent vasodilation.

In conclusion, L-NIL administered with lipopolysaccharide selectively decreases NO produced from iNOS but does not decrease endothelium-dependent and -independent vasodilation or alter HPV responses in isolated perfused rat lungs. Acetylcholine and bradykinin caused vasoconstriction in lipopolysaccharide-exposed lungs, which was probably a result of of endothelial dysfunction. In contrast to L-NAME, L-NIL attenuated the acetylcholine- and bradykinin-induced vasoconstriction. These results suggest that selective iNOS inhibition may have a protective role in the rat pulmonary circulation during sepsis.

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