L-Arginine prevents lung neutrophil accumulation and preserves pulmonary endothelial function after endotoxin

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L-Arginine prevents lung neutrophil accumulation and preserves pulmonary endothelial function after endotoxin. Am. J. Physiol. 274 (Lung Cell. Mol. Physiol. 18); L337–L342, 1998.—L-Arginine supplementation has been shown to restore endothelium-derived nitric oxide (NO) production in several pathological states. The purpose of this study was to examine the effect of administration of exogenous L-arginine on the endotoxin-induced lung neutrophil accumulation and impairment of endothelium-dependent guanosine 3′,5′-cyclic monophosphate (cGMP)-mediated pulmonary vasorelaxation in rats. Endothelium-dependent relaxation was tested by receptor-dependent [acetylcholine (ACh)] and receptor-independent (A-23187) pathways. Endothelium-independent relaxation was tested with sodium nitroprusside (SNP). In isolated pulmonary arterial rings, concentration-response curves were generated with ACh, A-23187, and SNP (10⁻⁹ to 10⁻⁶ M) 4 h after endotoxin (500 µg/kg ip) with and without prior administration of L-arginine (300 mg/kg ip). Lung neutrophil accumulation was determined by myeloperoxidase (MPO) assay. After endotoxin, lung neutrophil accumulation was significantly increased (MPO activity, 3.8 ± 0.4 vs. 0.8 ± 0.1 units/g lung weight in control cells; P < 0.05), which was prevented by L-arginine treatment (MPO activity, 1.3 ± 0.3 units/g lung weight; P < 0.05 vs. endotoxin). Endotoxin produced a significant impairment of endothelium-dependent cGMP-mediated pulmonary vasorelaxation by receptor-dependent (ACh) and receptor-independent (A-23187) pathways as well as of endothelium-independent relaxation (SNP). Prior treatment with L-arginine, but not with D-arginine, preserved endothelium-dependent vasorelaxation. Neither L- nor D-arginine influenced endotoxin-induced impairment of endothelium-independent, cGMP-mediated pulmonary vasorelaxation. We conclude that administration of exogenous L-arginine prevents endotoxin-induced lung neutrophil accumulation and attenuates its associated impairment of endothelium-dependent, cGMP-mediated pulmonary vasorelaxation.

METHODS

All animals received humane care in compliance with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health.

Animal housing and acclimation. Male Sprague-Dawley rats (Sasco, Omaha, NE) weighing 250–350 g were quarantined in quiet, humidified, light-cycled rooms for 2–3 wk before use.

Experimental protocol. Awake, fed rats were administered endotoxin (Salmonella typhimurium, 500 µg/kg ip) that was freshly prepared in 0.9% saline (1 ml). Control rats received an equal volume of saline. The rats were provided water and rat chow ad libitum during the subsequent 4-h period. Pulmonary vasomotor control mechanisms were studied in...
isolated pulmonary arterial rings 4 h after endotoxin administration. Lung neutrophil accumulation was determined 4 h after endotoxin or saline injection. Exogenous L- or D-arginine (300 mg/kg ip) was given 30 min before endotoxin administration. cGMP-mediated pulmonary vasorelaxation and lung neutrophil accumulation were studied 4 h later. All arginine- and arginine+ endotoxin-treated rats remained conscious and ambulatory throughout the experimental time course.

Isolated pulmonary arterial ring preparation. The rats were anesthetized with pentobarbital sodium (50 mg/kg ip). A median sternotomy was performed, and heparin sulfate (500 USP) was injected into the right ventricular outflow tract. After removal of the heart and lungs en bloc, the right and left main pulmonary arteries were dissected out and placed in Earle's balanced salt solution (EBSS) at 4°C. Under dissecting microscope magnification, the surrounding tissue was dissected from the pulmonary arteries. The right and left main branch pulmonary arteries were then cut into rings, each ring 3-4 mm wide; two pulmonary arterial rings were obtained from each rat. Care was taken during this process to avoid endothelial injury. EBSS is a standard physiological salt solution and contains 1.80 mM CaCl₂, 0.83 mM MgSO₄ (anhydrous), 5.36 mM KCl, 116.34 mM NaCl, 0.40 mM Na₂HPO₄ (dibasic), 5.50 mM D-glucose, 19.04 mM NaHCO₃, and 0.03 mM phenol red Na salt (as a pH indicator). The pulmonary arterial rings were then placed on 11-ml steel wires and suspended in individual 10-ml organ chambers containing EBSS. The organ chambers were surrounded by water jackets and were continually warmed (37°C). Ring tension was determined by 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 with a MacLab data-interface module (ADI Instruments, Milford, MA) on a Macintosh IIdi computer (Apple Computer, Cupertino, CA). Each organ chamber had a continual bubbling gas flow at 40 ml/min of 21% oxygen-5% carbon dioxide-74% nitrogen. This produced a PO₂ of 100–110 mmHg and a pH of ~7.4.

Pulmonary vasorelaxation by cGMP-mediated mechanisms. Cumulative concentration-response curves were generated for ACh, A-23187, and SNP. The optimal resting mechanical tension for the pulmonary arterial rings of this size was determined in a prior study (7) to be 750 mg. The rings were suspended at 750 mg and allowed to reach a steady state for 1 h, during which time the EBSS was changed every 15 min. A given ring was preconstricted with phenylephrine (PE) to achieve a PE-induced ring tension between 200 and 15 min. A given ring was preconstricted with phenylephrine (PE) to achieve a PE-induced ring tension between 200 and 150 mg. Cumulative concentration-response curves were then generated over the concentration range of 10⁻⁴ to 10⁻⁶ M ACh, A-23187, and SNP. For determination of the concentration-response curve, the ring was allowed to reach a steady state, usually requiring 2-3 min, before it advanced to the next highest concentration. The ring tension remaining in the rings in response to each dose of vasorelaxing agent was expressed in milligrams of PE-induced tension. Ten rings from five rats were studied in all groups.

MPO assay. The rats were anesthetized with pentobarbital sodium (50 mg/kg ip), and a median sternotomy was performed. The rats were heparinized (500 USP) by injection into the right ventricular outflow tract. After endotoxin or saline injection, lung MPO was determined such that 1 unit of MPO activity is the amount of enzyme that will reduce 1 μmol peroxidase/min (10). Lung wet-to-dry weight ratio was used as a parameter of lung water accumulation (1). Lung dry weight (mg) divided by lung wet weight (mg) x 100 = lung dry weight ratio. Control rats were studied 4 h after saline injection, and endotoxin-treated rats were studied 4 h after endotoxin injection. Lung wet weight was determined immediately after removal of the lungs. Lung dry weight was determined after the lungs had been kept for 72 h in an anhydrous oven.

Reagents. Standard reagents were obtained from Sigma (St. Louis, MO) with the exception of A-23187 that was from Calbiochem (La Jolla, CA). Fresh solutions were prepared daily with either deionized water or normal saline 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 650 computer and StatView software (Brain Power, Calabasas, CA). Data are presented as means ± SE of the number of pulmonary rings studied at each point of data collection. Statistical evaluation utilized a standard one-way analysis of variance with a post hoc Bonferroni-Dunn test. A P value of ≤0.05 was accepted as statistically significant.

RESULTS

Lung neutrophil accumulation and lung wet-to-dry weight ratio. As shown in Fig. 1, lung MPO in saline-injected rats was 0.8 ± 0.5 units/g lung weight. Endotoxin produced a significant increase in lung neutrophil accumulation (lung MPO 3.8 ± 0.4 units/g lung tissue after endotoxin alone; P < 0.05 vs. saline-injected control rats). However, L-arginine but not D-arginine prevented endotoxin-induced lung neutrophil accumulation (lung MPO 1.3 ± 0.3 units/g lung tissue after endotoxin + L-arginine; P < 0.05 vs. endotoxin alone). After endotoxin + D-arginine injection, lung MPO was 3.2 ± 0.2 units/g (P < 0.05 vs. saline control and L-arginine+endotoxin-treated rats but not different from endotoxin alone-treated rats).

The lung wet-to-dry weight ratio was not increased after this dose of endotoxin. The lung wet-to-dry weight ratio was 4.3 ± 0.4 in control rats (n = 4) and 4.2 ± 0.12 after endotoxin (n = 4).

Influence of exogenous L- or D-arginine on cGMP-mediated pulmonary vasorelaxation. Neither L- nor D-arginine alone influenced the concentration-response curves to ACh, A-23187, or SNP generated in pulmonary arterial rings of this size.
Endothelium-dependent, receptor-independent cGMP-mediated pulmonary vasorelaxation was also significantly impaired after endotoxin treatment. Pulmonary arterial rings from rats treated with L-arginine were significantly less impaired after endotoxin treatment compared with endotoxin alone-treated rats (Fig. 2B). Control rings were preconstricted to 303 ± 23 mg of PE-induced tension and relaxed to 11 ± 6 mg of tension with A-23187 (10⁻⁶ M). In endotoxin-treated rats preconstricted to 266 ± 23 mg of tension, 106 ± 15 mg of PE-induced tension remained in response to A-23187 (10⁻⁶ M). Thus L-arginine+endotoxin-treated rats had an impaired response to A-23187 compared with control rats (P < 0.05). However, L-arginine+endotoxin-treated rats had significantly less impairment of cGMP-mediated pulmonary vasorelaxation in response to ACh than endotoxin alone-treated rats (P < 0.05).

Endothelium-dependent cGMP-mediated vasorelaxation by direct stimulation of guanylate cyclase (response to SNP) was only mildly impaired after endotoxin alone. The L-arginine+endotoxin-treated rats had a response to direct guanylate cyclase stimulation similar to that of the endotoxin alone-treated rats. As shown in Fig. 3, control rings were preconstricted to 298 ± 14 mg of PE-induced tension and relaxed to 0 mg of tension with SNP (10⁻⁶ M). In rings from endotoxin-treated rats preconstricted to 275 ± 21 mg of tension, 18 ± 8 mg of PE-induced tension remained in response to SNP (10⁻⁶ M; P < 0.05 vs. control rings). Rings from L-arginine+endotoxin-treated rats were preconstricted with PE to 290 ± 19 mg of tension, with 18 ± 7 mg of tension remaining in response to SNP (10⁻⁶ M). Thus L-arginine+endotoxin-treated rats had a response to SNP that was not different from that of endotoxin alone-treated rats.

Endothelin-induced impairment of endothelium-dependent, receptor-dependent and -independent, cGMP-mediated pulmonary vasorelaxation was not different with d-arginine supplementation. As shown in Figs. 2 and 3, d-arginine failed to prevent endothelin-induced impairment of the response to ACh, A-23187, and SNP.

**DISCUSSION**

The results of this study demonstrate that administration of exogenous L-arginine before endotoxin prevented endotoxin-induced lung neutrophil accumula-
tion and attenuated the associated impairment of endothelium-dependent cGMP-mediated pulmonary vascular smooth muscle relaxation. Administration of exogenous L-arginine relatively preserved endothelium-dependent pulmonary vasorelaxation by receptor-dependent and -independent pathways, both of which require synthesis of NO. Endotoxin-induced impairment of endothelium-independent pulmonary vasorelaxation was unaffected by administration of exogenous L-arginine.

Endotoxin produces a lung injury in rats that is characterized by increased pulmonary vascular resistance, increased lung neutrophil accumulation, increased lung albumin leakage, and impairment of mechanisms of pulmonary vascular smooth muscle relaxation (3, 7, 12, 19, 27). In the present study, administration of exogenous L-arginine preserved endothelium-dependent cGMP-mediated pulmonary vascular smooth muscle relaxation after endotoxin. This effect of L-arginine on pulmonary endothelial function may be neutrophil independent, but it was associated with the finding that administration of exogenous L-arginine prevented lung neutrophil accumulation. The dose of L-arginine used in the present study (300 mg/kg) has been previously shown to abrogate systemic hypertension in spontaneously hypertensive rats. This effect was attributed to the ability of exogenous L-arginine administration to stimulate cGMP production via the L-arginine-NO pathway by providing NO synthase (NOS) with substrate (4). In the present study, administration of D-arginine failed to prevent endotoxin-induced lung neutrophil accumulation or pulmonary vasomotor dysfunction. It therefore seems probable that the effects of exogenous L-arginine administration seen in the present study are attributable to its role as a substrate for NOS.

L-Arginine has recently been identified as a critical regulator of vascular endothelial NO production. As the exclusive amino acid precursor of endogenous NO production (13, 21), it is transported into pulmonary vascular endothelial cells via carrier-mediated sodium-
transport pathways. In the absence of vascular injury, l-arginine is not usually felt to be a rate-limiting factor in endothelial production of NO; the intracellular concentration of l-arginine in cultured endothelial cells is reported to be well above the Michaelis-Menten constant for NOS, the concentration at which the reaction velocity of NOS is half maximal. For example, under normoxic conditions in a noninjured lung, McQueston et al. (18) demonstrated that l-arginine neither sustained nor potentiated ACh-induced pulmonary vasorelaxation. But in states of vascular injury including endotoxemia, l-arginine may become rate limiting in endothelium-dependent vasorelaxation. The reasons for this are unclear. Metabolism of l-arginine may be increased after endotoxin; Kurrek et al. (15) demonstrated that l-arginine metabolism to L-citrulline is increased after endotoxin pretreatment in an isolated perfused rat lung. Raij (22) found plasma levels of l-arginine decreased after endotoxin. Alternatively, the ability of l-arginine to function as a substrate for NOS may be impaired by circulating NOS inhibitors (28). Another possibility is that after endotoxin a critical relationship between l-arginine and other intracellular substances such as l-glutamine is disturbed (1).

Having recognized the importance of l-arginine in NO production in states of vascular injury, several investigators have shown that administration of exogenous l-arginine attenuates vasomotor dysfunction in several models of vascular injury. Nakanishi et al. (20) demonstrated that intracoronary l-arginine treatment during reperfusion improved endothelial function and diminished infarct size in dogs. In a piglet model of group B streptococcal sepsis, an l-arginine infusion lowered pulmonary arterial pressure and pulmonary vascular resistance (24). Girerd et al. (8) demonstrated that administration of exogenous l-arginine normalized endothelium-dependent vasorelaxation in hindlimb vessels of cholesterol-fed rabbits. In a rat model of salt-sensitive hypertension, Chen and Sanders (4) demonstrated that administration of l-arginine increased production of NO and abrogated systemic hypertension. Taken together, these studies suggest that administration of exogenous l-arginine restores endothelium-derived NO production in the pathological disorders in which it is diminished. In the present study, administration of exogenous l-arginine significantly attenuated endotoxin-induced impairment of two endothelium-dependent cGMP-mediated mechanisms (receptor dependent and independent) of pulmonary vascular smooth muscle relaxation.

Studies such as these implicate intracellular depletion of l-arginine as a potential mechanism underlying the impaired release of NO. In in vitro studies, release of NO from aortic endothelial cells cultured in l-arginine-deficient medium was enhanced by the addition of exogenous l-arginine (21). In isolated bovine pulmonary arterial rings depleted of l-arginine, exogenous l-arginine restored endothelium-dependent relaxation (9). In in vivo studies, exogenous administration of l-arginine normalized endothelial function in hypercholesterolemic animals in which the production and release of NO by endothelial cells may have been reduced (8, 23). In normal vessels, however, addition of l-arginine does not increase vasorelaxation because a sufficient amount of l-arginine is present to ensure saturation of NOS (23). In the present study, the addition of l-arginine alone had no effect on the concentration-response curves of either of the endothelium-dependent vasorelaxing agonists (ACh and A-23187), both of which require endothelial synthesis of NO to produce pulmonary vascular smooth muscle relaxation (Table 1).

Several investigators have demonstrated the importance of endothelium-derived NO as an important defense mechanism that protects the vascular endothelium from neutrophil-mediated injury. Using cat coronary arterial rings subjected to ischemia-reperfusion, Lefer and Ma (16) and Ma et al. (17) found an association between decreased basal NO production from cat coronary arterial rings and increased neutrophil adhesion to the coronary endothelium. These results implicate endothelial injury and the resultant loss of basal NO production in the development of pathological vasoconstriction after vascular injury. Carey et al. (2) and Siegfried et al. (26) have also demonstrated attenuation of vascular endothelial cell dysfunction after ischemia-reperfusion using NO-donating compounds. Kubes et al. (14) reported that leukocyte adherence was increased in the feline mesenteric artery after the administration of inhibitors of NO production (N-nitro-l-arginine methyl ester and N-monomethyl-l-arginine), suggesting that endothelium-derived NO may be an important modulator of leukocyte adherence. Last, inhaled NO has been shown to prevent lung neutrophil accumulation and the associated impairment of endothelium-dependent pulmonary vasorelaxation in acute lung injury produced by mesenteric ischemia-reperfusion (6).

Neutrophils contribute to endotoxin-induced impairment of cGMP-mediated pulmonary vascular smooth muscle relaxation (25). The results of the present study demonstrate that administration of exogenous l-arginine prevents endotoxin-induced lung neutrophil accumulation. This prevention of endotoxin-induced lung neutrophil accumulation was associated with a relative preservation of endothelium-dependent cGMP-mediated pulmonary vascular smooth muscle relaxation.

This work was supported by National Heart, Lung, and Blood Institute Grant R29-HL-49398 (to D. A. Fullerton) and an American College of Surgeons Faculty Research Grant (to R. C. McIntyre, Jr.).

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Received 1 October 1996; accepted in final form 25 November 1997.

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L-ARGININE ATTENUATES PULMONARY VASOMOTOR DYSFUNCTION


