Effects of endogenous and exogenous catecholamines on LPS-induced neutrophil trafficking and activation

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Abramham, Edward, Debra J. Kaneko, and Robert Shenkar. Effects of endogenous and exogenous catecholamines on LPS-induced neutrophil trafficking and activation. Am. J. Physiol. 276 (Lung Cell. Mol. Physiol. 20): L1–L8, 1999.—Endotoxemia produces elevations in catecholamine levels in the pulmonary and systemic circulation as well as rapid increases in neutrophil number and proinflammatory cytokine expression in the lungs. In the present experiments, we examined the effects of endogenous and exogenous adrenergic stimulation on endotoxin-induced lung neutrophil accumulation and activation. Levels of interleukin (IL)-1β, tumor necrosis factor (TNF)-α, and macrophage inflammatory protein (MIP)-2 mRNAs were increased in lung neutrophils from endotoxemic mice compared with those present in lung neutrophils from control mice or in peripheral blood neutrophils from endotoxemic or control mice. Treatment with the β-adrenergic antagonist propranolol before endotoxin administration did not affect trafficking of neutrophils to the lungs or the expression of IL-1β, TNF-α, or MIP-2 by lung neutrophils. Administration of the α2-adrenergic antagonist phenylephrine before endotoxia did not alter neutrophil accumulation as measured by myeloperoxidase (MPO) levels but did result in significant increases in IL-1β, TNF-α, and MIP-2 mRNA expression by lung neutrophils compared with endotoxia alone. Administration of the α2-adrenergic agonist phenylephrine before endotoxin did not affect trafficking of neutrophils to the lungs but was associated with significantly increased expression of TNF-α and MIP-2 mRNAs by lung neutrophils compared with that found after endotoxin alone. In contrast, treatment with the α2-adrenergic agonist UK-14304 prevented endotoxin-induced increases in lung MPO and lung neutrophil cytokine mRNA levels. The suppressive effects of UK-14304 on endotoxin-induced increases in lung MPO were not affected by administration of the nitric oxide synthase inhibitor N-nitro-L-arginine methyl ester. These data demonstrate that the initial accumulation and activation of neutrophils in the lungs after endotoxia can be significantly diminished by α2-adrenergic stimulation. Therapy with α2-adrenergic agents may have a role in modulating inflammatory pulmonary processes associated with sepsis-induced acute lung injury.

Lipopolysaccharide, α-adrenergic; β-adrenergic; acute lung injury; propranolol; phenylephrine; UK-14304; phenylephrine; nitric oxide

ACUTE INFLAMMATORY LUNG INJURY occurs frequently in patients with severe infections (1, 16). Massive accumulation of neutrophils in the lungs and increased pulmonary immunoregulatory cytokine levels are major characteristics of this condition (7, 16, 19, 46). Proinflammatory cytokines and chemokines, including interleukin (IL)-1β, tumor necrosis factor (TNF)-α, IL-8, and macrophage inflammatory protein (MIP)-2, can be produced by resident pulmonary cell populations, including alveolar macrophages and vascular endothelium (22, 29, 36, 47). However, recent data (32) indicate that neutrophils are also a source of IL-1β in the lungs after endotoxia and other pathophysiological conditions, such as hemorrhage, associated with the development of acute lung injury. Identification of lung neutrophils as a significant intrapulmonary source of IL-1β after endotoxia may be particularly important because several studies (34, 42) have shown that IL-1β is a major proinflammatory cytokine in bronchoalveolar lavage fluids obtained from patients with acute lung injury.

Endotoxia and sepsis are associated with the release of high levels of catecholamines into the pulmonary and systemic circulation (40). Catecholamines, often in large doses, are frequently administered to critically ill septic and endotoxemic patients to maintain blood pressure and cardiac function (33). In addition to their vasoactive effects, catecholamines can affect expression of proinflammatory cytokines (18, 33, 44). For example, TNF-α production by lipopolysaccharide (LPS)-stimulated macrophages is augmented by α-adrenergic (43) and decreased by β-adrenergic agonists (37). In human volunteers, endotoxin-induced increases in serum TNF-α levels were diminished when pretreatment with epinephrine was provided (48, 49).

Even though epinephrine has mixed β- and α-adrenergic agonist properties, in vitro studies of human peripheral blood mononuclear cells suggested that the β- but not the α-adrenergic agonist properties of epinephrine were responsible for the inhibition of TNF-α release (37). However, no studies have specifically examined the ability of catecholamines to affect cytokine production by lung neutrophils.

There is evidence that catecholamines can directly affect adhesion of leukocytes to endothelial surfaces (4, 6). In particular, β-adrenergic stimulation of endothelial cells has been shown to inhibit neutrophil adhesion (5, 10). Therefore, catecholamines, whether produced endogenously or administered exogenously, may be capable of affecting the development and progression of acute lung injury. Such effects may occur through modulating proinflammatory cytokine production by neutrophils or other pulmonary cell populations, through direct effects on neutrophil-endothelial interactions, or through both mechanisms. To examine these issues, we treated endotoxemic mice with α- and β-adrenergic antagonists or agonists and then determined...
whether such adrenergic manipulation affected either the accumulation of neutrophils in the lungs or the expression of proinflammatory cytokines by lung neutrophils.

**METHODS**

**Materials.** Escherichia coli 0111:B4 endotoxin, phenylephrine, UK-14304 (brimonidine), DMSO, collagenase, and DNase (type I) were obtained from Sigma (St. Louis, MO). RPMI 1640 medium (with 10 mM HEPES and 20 mM l-glutamine), used in the cell isolation procedures, was obtained from BioWhittaker Products (Walkersville, MD). Fetal calf serum (FCS) and penicillin-streptomycin were purchased from Gemini Bioproducts (Calabasas, CA). Percoll was obtained from Pharmacia (Uppsala, Sweden). For the RNA extractions, guanidinium and phenol-chloroform (5:1, pH 4.7) were purchased from Fisher Scientific (Pittsburgh, PA) and isopropanol was purchased from Sigma. Taq DNA polymerase was obtained from Perkin-Elmer (Branchburg, NJ), and the cytokine primers for IL-1b and TNF-a were from Clontech (Palo Alto, CA). Primers for MIP-2 were synthesized by Operon Technologies (Alameda, CA) with sequences kindly provided by Dr. David Baltimore (Massachusetts Institute of Technology, Cambridge, MA). Potassium phosphate, hexadecyltrimethylammonium bromide, hydrogen peroxide, and O-dianisidine used in the myeloperoxidase (MPO) assays were obtained from Sigma.

**Animals.** Male BALB/c mice (7–8 wk old) were purchased from Harlan Sprague Dawley (Indianapolis, IN). Animals were kept on a 12:12-h light-dark cycle and given free access to food and water. Studies were conducted when the mice were between 8 and 12 wk of age.

**Experimental model of endotoxemia.** A murine model of endotoxemia was used as previously reported (32). Briefly, mice were given intraperitoneal injections of 25 mg/kg of LPS (E. coli 0111:B4) in a volume of 0.2 ml of phosphate-buffered saline (PBS). This dose of LPS has previously been shown to cause lung injury 24 h postinjection (12, 13).

In designated experiments, mice were treated intraperitoneally with the p-adrenergic antagonist phentolamine (10 mg/kg) or the p-adrenergic antagonist propranolol (3 mg/kg) 30 min before LPS administration. These doses of phentolamine and propranolol have been used previously by our laboratory and result in complete p- and p-adrenergic blockade (26). To investigate the effects of p-adrenergic-specific agonists, either phenylephrine (p-specific) or UK-14304 (p-specific) at 1 mg/kg was administered intraperitoneally 30 min before LPS treatment. Phenylephrine was resuspended in PBS, whereas UK-14304 was dissolved in DMSO at 10 mg/ml and then diluted to 1 mg/kg dose in PBS. All drugs were administered in a volume of 0.2 ml. The following control groups were included: normal (unmanipulated), dilute DMSO, dilute DMSO-LPS, phenylephrine only, UK-14304 only, propranolol only, and phentolamine only.

**Isolation of intraparenchymal pulmonary mononuclear cells and neutrophils.** Intraparenchymal pulmonary mononuclear cells and neutrophils were isolated with techniques previously described by our laboratory (2). In brief, after the mouse was euthanized by cervical dislocation under methoxyflurane anesthesia, the chest was opened and the lung vascular bed was flushed by injecting 3–5 ml of cold PBS through the right ventricle of the heart. The lungs were then removed and rinsed two times in ice-cold RPMI 1640 medium. The lungs from each mouse were finely minced and placed in RPMI 1640 medium containing 10 mM HEPES, 20 mM l-glutamine, 5% FCS, 1% penicillin-streptomycin, 20 U/ml of collagenase, and 1 mg/ml of DNase. After incubation for 60 min at 37°C, tissue fragments were forced three times through a 21-gauge needle to disrupt any remaining intact tissue. Tissue fragments and dead cells were removed by rapid filtration on a glass wool column. The resulting cells were collected by centrifugation.

**Neutrophil isolation.** Neutrophil isolation from peripheral blood as well as from intraparenchymal pulmonary mononuclear cell and neutrophil populations was performed with a modification of the technique described by Sugawara et al. (45) and previously used in our laboratory (32). To isolate peripheral blood neutrophils, mice were anesthetized with methoxyflurane and then exsanguinated. Blood was withdrawn by cardiac puncture and collected into 5 U of heparin. The collected blood was then mixed into 2 volumes of chilled PBS and layered onto a gradient of 5 ml each of 1.097 and 1.085 g/ml of Percoll. To isolate lung neutrophils, the intraparenchymal pulmonary mononuclear and neutrophil cell pellet was resuspended in 1 ml of PBS and layered on a gradient of 5 ml each of 1.097 and 1.085 g/ml of Percoll. After centrifugation at 600 g for 25 min at 18°C, the neutrophil-rich fraction was collected from the gradient interface and washed with RPMI 1640 medium. Viability as determined by trypan blue exclusion was consistently >98%. The purity of the isolated neutrophil populations was assessed for each experiment by Wright staining of cytospin preparations and was as great as 98%.

**Isolation of alveolar macrophages.** Alveolar macrophages were isolated as previously described (38). In brief, bronchoalveolar lavage fluid was collected either 10 ml of PBS was injected and then aspirated intratracheally three times. Alveolar macrophages were obtained by centrifugation of bronchoalveolar lavage samples. Macrophage purity and viability, as assessed by trypan blue exclusion and cytospin preparations, were consistently >98%.

**MPO assay.** MPO assays were performed essentially as described by Goldblum et al. (15). In brief, red blood cells were flushed from the lung vascular bed by an injection of 5 ml of iced PBS through the right ventricle. The lungs were removed, rinsed two times in iced PBS, blotted dry, and snap-frozen in liquid nitrogen. The frozen lungs were then weighed and stored at –80°C if not used immediately. Frozen tissue was homogenized in 20 mM potassium phosphate buffer (pH 7.4) and then centrifuged for 30 min at 20,000 g, and the resulting pellet was resuspended in 50 mM potassium phosphate buffer containing 0.5% hexadecyltrimethylammonium bromide. Samples were sonicated and placed at 60°C for 2 h. After incubation, 1 ml of each sample was centrifuged briefly, and the supernatant was assayed for MPO activity in a hydrogen peroxide-O-dianisidine buffer via spectrophotometric analysis at 420 nm. Results are given as units of MPO activity per gram of lung tissue.

**Semiquantitative PCR.** The basic procedure used for semiquantitative PCR has been described previously by our laboratory (38). Groups of six mice, with PCR results obtained from individual mice, were used for each experimental condition. In brief, after purified neutrophil populations had been lysed in 4 M guanidinium thiocyanate-25 mM sodium citrate-0.5% sarcosyl-0.1 M 2-mercaptoethanol, mRNA was phenol extracted following the method of Chomczynski and Sacchi (8). cDNA was synthesized from the mRNA from 100,000 cells/sample with Moloney murine leukemia virus reverse transcriptase and random hexamer oligonucleotide primers as described by Kawasaki et al. (21). Semiquantitative PCR was performed with the cDNA from 1,000 cells/sample. A single PCR master mix was prepared, and aliquots were used as samples in all treatment groups for each experiment. After an initial 4-min denaturation step at 95°C, between 20 and 40 cycles of PCR were carried out as follows: 1 min, 95°C denaturation; 1 min, 60°C anneal; and 1 min, 72°C extension. Coamplification of the housekeeping genes hypoxanthine phosphoribosyl transferase (HPRT) and glyceraldehyde-3-
phosphate dehydrogenase was used to standardize the PCR products. PCR products were visualized by electrophoresis on 1.6% agarose gels stained with ethidium bromide. The number of PCR cycles was selected for the cytokine product so that the ethidium bromide-stained amplified DNA products were between barely detectable and below saturation levels. For analysis, the gel image was photographed and scanned with a gel-documentation system (ImageStore 5000 with GelBase Windows Software, Ultraviolet Products, San Gabriel, CA).

Results for each cytokine were normalized to those for HPRT or glyceraldehyde-3-phosphate dehydrogenase.

Statistical analysis. Because of inherent variability between groups of mice for each experimental condition, the entire group of animals was prepared and studied at the same time. For each experimental condition, mice in all groups had the same birth date and had been housed together. For semiquantitative PCR, cells were obtained individually from each animal and analyzed individually before group data were calculated. All experiments were repeated two or three times with separate, additional groups of animals. Data are presented as means ± SE for each experimental group. Comparisons between groups were performed by one-way ANOVA and the Student-Newman-Keuls test. A P value of < 0.05 was considered significant.

RESULTS

Neutrophil migration and activation in response to endotoxin. Lung MPO levels increased >16-fold from baseline within 1 h of endotoxin administration (Fig. 1). The neutrophils that were present in the lungs after endotoxemia showed significant increases in mRNA levels of IL-1β, TNF-α, and MIP-2 compared with lung neutrophils from control, unmanipulated mice (Fig. 2). In peripheral blood neutrophils isolated at the same time point after endotoxemia, levels of mRNA for TNF-α were increased, but there were no significant changes in the amounts of mRNA for IL-1β and MIP-2 compared with those in control, unmanipulated mice (Fig. 2). However, for all three cytokines examined, mRNA levels after endotoxemia were greater in lung neutrophils than in peripheral blood neutrophils. No differences in the levels of mRNA for IL-1β, TNF-α, and MIP-2 were found in alveolar macrophages collected 1 h after endotoxin administration compared with those from control animals (Fig. 3).

Effects of endotoxin-induced catecholamine release on lung neutrophil accumulation and activation. To examine the role of endogenous catecholamine release on neutrophil accumulation in the lungs after endotoxemia, we pretreated mice with either the nonspecific α-adrenergic antagonist phentolamine or the nonspecific β-adrenergic antagonist propranolol. Neither propranolol nor phentolamine pretreatment produced any significant change in lung MPO concentrations compared with those present in mice given endotoxin alone (Fig. 4).

We also examined the effects of α- or β-adrenergic blockade on endotoxin-induced alterations of proinflam-
Inflammatory cytokine expression in lung neutrophils (Fig. 5). Pretreatment with the β-adrenergic antagonist propranolol did not affect endotoxin-induced increases in IL-1β, TNF-α, or MIP-2 mRNA expression in lung neutrophils. However, α-adrenergic blockade with phentolamine was associated with up to threefold greater levels of mRNA for each of the proinflammatory cytokines studied. Propranolol or phentolamine given alone without endotoxin administration did not have any effect on lung MPO content. MPO levels with the drug alone were comparable to those seen in control, unmanipulated mice (data not shown). A previous study by our laboratory (26) also showed that these drugs had no effect on baseline levels of cytokine mRNA expression in intraparenchymal pulmonary mononuclear and neutrophil cell populations.

Effects of exogenous α1-adrenergic stimulation on lung neutrophil accumulation. To explore more completely α-adrenergic-mediated effects on endotoxin-induced lung neutrophil accumulation and activation, we pretreated mice with either the α1-adrenergic agonist phenylephrine or the α2-adrenergic agonist UK-14304 (Fig. 6). Pretreatment with phenylephrine had no effect on endotoxin-induced increases in lung MPO concentrations. However, administration of UK-14304 before endotoxin decreased lung MPO content to levels not significantly different from those seen in unmanipulated mice not given endotoxin.

Administration of vehicle, phenylephrine, or UK-14304 without endotoxin did not alter baseline lung MPO concentrations from levels seen in control, unmanipulated mice (data not shown). Likewise, administration of vehicle did not affect LPS-induced increases in lung MPO content (data not shown).

Increased production of nitric oxide (NO) can attenuate neutrophil adhesion to endothelial surfaces (3, 24). Because α2-adrenergic stimulation can enhance NO production by vascular endothelial cells (27, 51), we examined whether such effects on NO generation contributed to the observed suppression by UK-14304 of endotoxin-induced neutrophil accumulation in the lungs.

Administration of the nonspecific NO synthase inhibitor N-nitro-L-arginine methyl ester before endotoxin injection did not significantly affect lung MPO (Fig. 7). Similarly, N-nitro-L-arginine methyl ester administration did not modify the suppressive effects of UK-14304 on lung MPO levels after LPS administration.

Effects of exogenous α-adrenergic stimulation on lung neutrophil activation. To examine the effects of α-adrenergic stimulation on endotoxin-induced increases in
proinflammatory cytokine mRNA expression by lung neutrophils, we pretreated mice with either the α1-adrenergic agonist phenylephrine or the α2-adrenergic agonist UK-14304 (Fig. 8). Under these conditions, UK-14304 inhibited endotoxin-induced increases in mRNA levels of IL-1β, TNF-α, and MIP-2 in lung neutrophils. With UK-14304 pretreatment, the levels of TNF-α and MIP-2 mRNAs in endotoxemic animals were not different from those present in control, unmanipulated mice. IL-1β mRNA levels in lung neutrophils

Fig. 5. Effects of α- and β-adrenergic blockade on LPS-induced increases in cytokine mRNA expression. mRNA levels of IL-1β (A), TNF-α (B), and MIP-2 (C) were measured in lung neutrophils isolated from C mice, mice given LPS 1 h previously, or mice treated with either PRP or PHT before LPS administration. Results were normalized to HPRT and are means ± SE; n = 4 or 5 mice/group. For all 3 cytokines studied, PRP did not affect LPS-induced increases in mRNA levels, whereas PHT treatment resulted in further increases above those seen in mice treated with LPS alone. **P < 0.01 vs. C. ***P < 0.001 vs. C. +++P < 0.001 vs. LPS. +++P < 0.001 vs. LPS. D: a representative experiment showing amplified cytokine and hypoxanthine phosphoribosyl transferase (HPRT) gene product.

Fig. 6. Effects of α1- and α2-adrenergic agonists on lung MPO content. MPO levels were assayed in lungs from C mice, from mice given LPS, or from mice pretreated with α1-adrenergic agonist phenylephrine (PHE) or α2-adrenergic agonist UK-14304 (UK) before LPS administration (PHE/LPS and UK/LPS, respectively). Results are means ± SE; n = 6 mice/group. α2-Adrenergic-specific stimulation with UK significantly reduced lung MPO to levels comparable with C animals. ***P < 0.001 vs. C. +++P < 0.001 vs. UK/LPS.

Fig. 7. Effects of N-nitro-L-arginine methyl ester (LN) and UK on lung MPO content. MPO was measured in lungs of C mice or mice treated with LN alone, LPS alone, LN before LPS (LN/LPS), UK before LPS (UK/LPS), or LN and UK before LPS (LN/UK/LPS). Results are means ± SE; n = 5 mice/group. ***P < 0.001 vs. C, LN, UK/LPS, and LN/UK/LPS.
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Fig. 8. Effects of α1- and α2-adrenergic agonists on LPS-induced increases in IL-1β (A), TNF-α (B), and MIP-2 (C) mRNA expression. Lung neutrophils were isolated from C mice, mice given LPS 1 h previously (LPS), and mice treated with either α1-adrenergic agonist PHE or α2-adrenergic agonist UK before LPS administration. Results were normalized to HPRT and are means ± SE; n = 5 or 6 mice/group. *P < 0.05 vs. C. **P < 0.01 vs. C. ***P < 0.001 vs. C. +++P < 0.001 vs. UK/LPS. #P < 0.05 vs. LPS. ##P < 0.01 vs. LPS.

from UK-14304-pretreated mice were also decreased compared with those present in animals given endotoxin alone but were greater than those in lung neutrophils from control, unmanipulated mice. By contrast, administration of phenylephrine before endotoxin did not reduce LPS-induced elevations in mRNA expression for any of the three cytokines studied and, in fact, produced increases in the amounts of mRNA for TNF-α and MIP-2 in lung neutrophils that were even greater than those found in endotoxemic mice not given other therapies.

Administration of vehicle alone or up to fivefold greater concentrations (i.e., 5 mg/kg) of phenylephrine or UK-14304 without endotoxin did not alter cytokine mRNA expression in lung neutrophils compared with the levels present in control, unmanipulated mice. Similarly, administration of vehicle did not affect LPS-induced elevations in cytokine mRNA expression (data not shown). Treatment with phenylephrine or UK-14304 in control, unmanipulated animals or before endotoxemia did not have any effect on IL-1β, TNF-α, or MIP-2 expression in peripheral blood neutrophils or alveolar macrophages (data not shown).

DISCUSSION

In the present study, we found that neutrophils rapidly accumulated in the lungs after endotoxemia and produced increased amounts of mRNA for proinflammatory cytokines including IL-1β, TNF-α, and MIP-2. Although blockade of endogenous α1- or β-adrenergic stimulation did not appear to influence the migration of neutrophils to the lungs, activation of these lung neutrophils to produce proinflammatory cytokines was affected by α-adrenergic blockade. In particular, inhibition of endogenous α-adrenergic effects with phenolamine resulted in further increases in the expression of IL-1β, TNF-α, and MIP-2 to levels greater than those seen in lung neutrophils isolated from mice treated with endotoxin alone. The suppressive effects of α-adrenergic stimulation on lung neutrophil activation appeared to be due to α2-adrenergic effects because enhanced α2- but not α1-adrenergic stimulation prevented endotoxin-induced increases in the expression of proinflammatory cytokines.

Signal transduction through α1-adrenergic receptors involves G proteins that activate phospholipase C, leading to hydrolysis of membrane phosphatidylinositols and increases in intracellular calcium levels (20, 28). Engagement of α2-receptors, through coupling to inhibitory G proteins, decreases adenylate cyclase-associated cAMP production (20, 28). Binding sites for the transcriptional regulatory factors nuclear factor-κB (NF-κB) (17, 41) and cAMP responsive element binding protein (CREB) (1, 9, 30, 52) are present in the promoter/enhancer regions of each of the cytokines examined in the present study. Increases in either intracellular Ca2+ or cAMP can activate NF-κB and CREB (14, 25, 35, 39). However, the results from the present experiments, showing relative dominance of α2-adrenergic effects on lung neutrophils after endotoxemia, would suggest that cAMP, probably through affecting protein kinase A-associated pathways (25, 39), is relatively more important under these conditions in modulating NF-κB- and/or CREB-associated proinflammatory cytokine transcription.

Previous studies (37, 43, 44) examined the effects of catecholamines on the systemic cytokine responses in vivo or the in vitro release of proinflammatory cytokines by macrophages. However, little information is available concerning interactions between catecholamines, pulmonary inflammatory processes, and neutrophils in the setting of endotoxemia. The addition of β-adrenergic agonists to LPS-stimulated macrophages or peripheral blood mononuclear cells inhibits IL-1, IL-6, and TNF-α production (23, 37, 50). These previous studies (37, 50) did not show that α-adrenergic stimulation had any significant suppressive effects on proinflammatory cytokine release by macrophage or mono-
nuclear cell populations. Treatment of endotoxemic mice with the specific α2-adrenergic antagonist CH-38083 decreased circulating TNF-α levels, but the mechanism of this effect appeared to be indirect, resulting from increased norepinephrine release and enhanced β1-adrenergic stimulation (11, 18).

In previous experiments by our laboratory (26), we found that α-adrenergic-receptor blockade prevented hemorrhage-induced increases in proinflammatory cytokine production by intraparenchymal lung cell populations, which included large numbers of neutrophils. In contrast, β-adrenergic blockade increased the amounts of mRNA for IL-1β and TNF-α to levels that were greater than those present in untreated, hemorrhaged mice (26). The results of the present experiments did not show similar inhibitory effects of β-adrenergic stimulation in lung neutrophils obtained after endotoxemia. Different adrenergic-associated mechanisms would therefore appear to be involved in lung neutrophil activation after hemorrhage or endotoxemia. In particular, although the present studies indicate that α2-adrenergic stimulation is inhibitory after endotoxemia, there is no evidence to suggest that similar regulatory mechanisms affect lung neutrophils after blood loss.

Although the present study demonstrated that lung neutrophils were activated to a greater extent after endotoxemia than were peripheral blood neutrophils, these results do not necessarily imply that neutrophil activation occurs in the lungs. In previous experiments by our laboratory (32), immunohistochemical studies found that the neutrophils present in the pulmonary vasculature as well as in the pulmonary parenchyma after endotoxemia stained positively for IL-1β, showing that activation could occur within the intravascular space. Although neutrophil activation may occur as a result of interaction with vascular endothelial cells in the lungs, it is also possible that extrapulmonary events may induce activated neutrophils to traffic rapidly to the lungs. Either of these potential mechanisms for neutrophil activation could be affected through α2-adrenergic effects. Because α2-adrenergic receptors are present on neutrophils (31), α2-adrenergic agonists may directly affect neutrophils in pulmonary or extrapulmonary intravascular sites. Alternatively, α2-adrenergic stimulation may downregulate expression of neutrophil costimulatory molecules on vascular endothelium, thereby decreasing neutrophil activation. Mechanisms involving α2-adrenergic-dependent effects on vascular endothelium would explain the link between the inhibition of neutrophil accumulation in the lungs and the activation seen with UK-14304 therapy. However, because inhibition of NO synthase did not alter the suppressive effects of α2-adrenergic stimulation on neutrophil accumulation in the lungs, alterations in NO production by the vascular endothelium do not appear to be involved in this process.

In the present experiments, no increase in the expression of IL-1β, TNF-α, or MIP-2 was present in alveolar macrophages isolated 1 h after endotoxin administration. These results are consistent with previous findings by our laboratory (32), where immunohistochemical studies showed increased staining for IL-1β in lung neutrophils and endothelial cells but not in alveolar macrophages at this same postendotoxemia time point. Although alveolar macrophages can be stimulated in vitro by endotoxin to produce proinflammatory cytokines (46), the present data suggest that such activation does not occur in vivo in the period immediately after endotoxemia.

Neutrophils, by producing proinflammatory cytokines and expressing cytotoxic products, appear to play an important role in the development and progression of acute lung injury (32). Inhibition of accumulation and/or activation of neutrophils in the lungs may therefore be important in improving the outcome from this condition. The present results suggest that administration of α2-adrenergic agonists may have therapeutic utility in clinical settings in which endotoxemia plays a major role, such as sepsis-induced acute respiratory distress syndrome.

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