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Attenuation of Lung Inflammation by Adrenergic Agonists in Murine Acute Lung Injury

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Background: **Acute lung injury leading to a systemic inflammatory response greatly increases mortality in critically ill patients. Cardiovascular management of these patients frequently involves β-adrenergic agonists. These agents may alter the inflammatory response. Therefore, the authors tested the hypothesis that β-adrenergic agonists alter the pulmonary inflammatory response during acute lung injury in mice.**

Methods: **Five-week-old CD-1 mice received continuous infusions of 10 $\mu g \cdot kg^{-1} \cdot min^{-1}$ dobutamine, 6 $\mu g \cdot kg^{-1} \cdot min^{-1}$ dopexamine, or vehicle *via* intraperitoneal mini osmotic pumps, followed immediately by intratracheal instillation of approximately 2 μg/kg endotoxin (or phosphate-buffered saline control). Six hours later the mice were killed, and lung lavage was performed. Interleukin-6 and -10 concentrations in lung homogenates were measured using enzyme-linked immunosorbent assay. Interleukin-6 and macrophage inflammatory protein-2 mRNA was measured using reverse-transcription polymerase chain reaction.**

Results: **Interleukin-6 protein and mRNA significantly increased after intratracheal endotoxin ($P < 0.001$), and the fraction of neutrophils in lung lavage fluid increased in endotoxin-treated (41 ± 25%) *versus* control mice (2 ± 4%, $P < 0.05$). Treatment of endotoxic mice with dobutamine significantly decreased interleukin-6 protein ($P < 0.05$) and mRNA ($P < 0.05$) expression. Dopexamine had similar but less pronounced effects. Dobutamine decreased interleukin-10 expression, whereas dopexamine did not. In endotoxemic mice, both dobutamine and dopexamine decreased induction of macrophage inflammatory protein-2 mRNA ($P < 0.05$) and reduced the fraction of neutrophils in lung lavage fluid ($P < 0.05$).**

Conclusions: **In endotoxin-induced acute lung injury, β-adrenergic agonists can significantly decrease proinflammatory cytokine expression, decrease induction of chemokine mRNA, and decrease the resultant neutrophil infiltrate in the lung.**

POSTOPERATIVE and nonsurgical acute lung injury is a common reason for admission to intensive care units. Acute lung injury leads to a systemic inflammatory response that can cause the acute respiratory distress syndrome (ARDS) and multiple organ failure.[1,2] The presence of ARDS or multiple organ failure increases the mortality of the underlying disease substantially.[3–5] Adrenergic agents, including α, β, and dopaminergic agonists, are commonly used in critically ill patients with ARDS and multiple organ failure for hemodynamic support by affecting cardiac output, vasomotor tone, and splanchnic blood flow.[6] More recent evidence shows that these vasoactive substances may have a direct effect on cytokine release and may influence the inflammatory response.[7] β-Adrenergic receptor agonists both in *in vitro* and *in vivo* experiments have diminished endotoxin-induced tumor necrosis factor α (TNF-α) production[8–11] and have attenuated the increase in permeability in the lungs and liver.[12] β_2-Adrenergic agonists also reduce hepatic cellular injury in a porcine model of sepsis.[13]

Whether β-Adrenergic agonists modify the pulmonary inflammatory response to acute lung injury is not known. Therefore, we tested the hypothesis that dobutamine and dopexamine alter the endotoxin-induced pulmonary inflammatory response, focusing on early proinflammatory cytokine expression, induction of chemokines, and subsequent leukocyte infiltration.

To accomplish this, we first determined the effect of dobutamine and dopexamine on endotoxin-induced early inflammatory cytokine expression in the lung. Specifically, we measured interleukin-6 as an important representative early proinflammatory cytokine[14] that, among many measured proinflammatory cytokines, is best correlated with clinical outcome of ARDS and multiple organ failure.[14–16] We chose to measure interleukin-10 as a representative antiinflammatory cytokine.[14] Proinflammatory cytokines induce chemotactic cytokine (chemokine) production. Therefore, we next looked for evidence of induction of chemokines in the lung by measuring macrophage inflammatory protein-2 (MIP-2) mRNA. MIP-2 is the murine C-X-C chemokine functionally analogous to human interleukin-8,[14] which is an important chemokine predicting severity and outcome in human ARDS.[17,18] Chemokines result in leukocyte infiltration in the lung. Therefore, we measured the percent neutrophils in bronchoalveolar lavage as a clinically relevant response to this pulmonary inflammatory response.

Methods

Animal Model

These experiments were approved by the University of British Columbia Animal Care Committee and conform to Canadian and National Institutes of Health guidelines regarding animal experimentation.

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All experiments were conducted in outbred 5-week-old female CD-1 mice given food and water *ad libitum*. Mice were anesthetized with inhaled 3% halothane while breathing spontaneously. Intraperitoneal pumps (Alzet, Newark, DE) delivering continuous infusions of dobutamine at 1 μl/h (10 $\mu g \cdot kg^{-1} \cdot min^{-1}$), dopexamine (6 $\mu g \cdot kg^{-1} \cdot min^{-1}$), or vehicle (5% dextrose to control for surgical manipulation) were inserted through a 1-cm midline abdominal incision that was closed in two layers using interrupted 5.0 silk sutures. These drug infusion doses have approximately equivalent hemodynamic effects[19] and were chosen to represent a moderate to high dose in humans to be relevant to clinical practice. For example, a 1,500 μg/kg single intraperitoneal injection of dobutamine in mice increased heart rate by approximately 25% and increased velocity of myocardial circumferential fiber shortening by 60%,[20] suggesting that our intraperitoneal infusion of 10 $\mu g \cdot kg^{-1} \cdot min^{-1}$ dobutamine was not excessively high. An intravenous infusion of 2 $\mu g \cdot kg^{-1} \cdot min^{-1}$ dobutamine in mice increased heart rate by approximately 80%,[21] suggesting that our intraperitoneal infusion of 10 $\mu g \cdot kg^{-1} \cdot min^{-1}$ dobutamine was not excessively low.

Five minutes after pump insertion and while mice were still anesthetized with 3% halothane, 40 μl of 1.5 mg/ml endotoxin (lipopolysacharide, Sigma Chemicals, Oakville, Ontario, Canada) in phosphate-buffered saline (PBS) or 40 μl of endotoxin-free PBS alone was instilled intratracheally. The mice were then allowed to recover in room air. Six hours after endotoxin or PBS instillation, the mice were anesthetized with 5% halothane and killed by rapid exsanguination by cardiac puncture. This 6-h time point was chosen based on preliminary studies that demonstrated that the maximal proinflammatory cytokine concentrations after endotoxin administration occurred at this time. In some mice, a lung lavage was performed using 1 ml PBS, and leukocyte differential counts were determined on Wright-stained cytospins. In other mice, the right lung was excised, frozen in liquid nitrogen, and stored at −70°C for subsequent enzyme-linked immunosorbent assay (ELISA) measurements while the left lung was used to measure mRNA using reverse-transcription polymerase chain reaction.

Cytokine Enzyme-linked Immunosorbent Assays

The lung samples were homogenized in 1 ml of ice-cold PBS and then centrifuged at 1,500 rpm for 10 min at 4°C. Entire right lungs were used for the ELISA measurements to allow comparison of equivalent samples between groups. The supernatant was subsequently stored at −20°C. Antigenic interleukin-6 and -10 concentrations were measured in lung homogenates using sandwich ELISAs (Pharmingen, San Diego, CA). The lower detection limit for both interleukin-6 and -10 ELISAs was 60 pg/ml. ELISA plates were incubated at 4°C overnight with 50 μl per well of desired capture antibody. Plates were washed four times, and nonspecific binding was blocked using 200 μl of PBS with 2% bovine serum albumin per well for 90 min. Plates were washed four times followed by incubation of 50 μl of diluted cell-free supernatant for 3 h at room temperature. The sample was replaced with 50 μl (1 μg/ml) of the paired biotynlated antibody and incubated for 60 min. Avidin-peroxidase conjugate was added (Bio-Rad Laboratories, Hercules, CA) followed by chromagen substrate (OPD; Dako, Mississauga, Ontario, Canada). Plates were read at 490 nm using an ELISA plate reader (Rainbow Reader; SLT Lab Instruments, Salzburg, Austria).

Reverse-Transcription Polymerase Chain Reaction

Total cellular RNA was isolated from snap frozen lungs by phenol chloroform extraction. RNA was ethanol precipitated and dissolved in diethyl pyrocarbonate–treated water, and total RNA concentration was determined by spectrophotometry. Five micrograms of RNA was reversed transcribed (SuperScript II reverse transcriptase; Gibco BRL, Burlington, Ontario, Canada) using oligo(dT)12-18 primers (Gibco BRL). The cDNA was amplified by PCR using specific primers for interleukin-6, MIP-2, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) using optimized protocols. Primers for interleukin-6 were 5' GAT GCT ACC AAA CTG GAT ATA ATC 3' and 5' GGT CCT TAG CCA CTC CTT CTG TG 3'.[22] Primers for MIP-2 were 5' GCT-GGC-CAC-CAA-CCA-CCA-GG 3' and 5' AGC-GAG-GCA-CAT-CAG-GTA-CG 3'. Primers for GAPDH, a reporter mRNA, were 5' CCC ATC ACC ATC TTC CAG 3' and 5' ATG ACC TTG CCC ACA GCC 3'. The reverse-transcribed cDNA (0.5 μg in 2 μl) was added with specific cytokine primer pairs to a PCR mix with 1 U Taq DNA polymerase (Gibco BRL) in a 20-μl reaction volume. PCR for interleukin-6 and GAPDH used 38 cycles of 95°C for 30 s, 60°C for 45 s, and 72°C for 30 s followed by one cycle of 72°C for 6 min. This number of cycles was chosen because all three PCRs were in their exponential phase of amplification. PCR products were identified by electrophoresis on a 2% agarose gel containing 0.2 μg of ethidium bromide per milliliter. The resulting image was captured (Eagle Eye; Stratagene, La Jolla, CA) and densitometry performed using an automated gel imaging system (Image PC; Scion Corporation, Frederick, MD).

Lung Wet Weight/Dry Weight Ratio

In a second identical series of experiments, the right lung was excised, blotted dry, and weighed to obtain wet weight. The lung was then dried at 60°C and weighed daily. After 48 h, no further change in weight was observed in any sample. This weight was taken as dry weight for the calculation of the wet weight/dry weight ratio.

Arterial and Mixed Venous Oxygen Saturation

In this second series of experiments, arterial blood was withdrawn from the left ventricle within 10 s of induction of anesthesia and thoracotomy at the end of the experiment. The mice were spontaneously breathing room air (fraction of inspired oxygen [F_{IO_2}] = 0.21) so that this measure reflects lung oxygen exchange. Oxygen saturation was measured using a co-oximeter (IL482; Instrumentation Laboratories, Lexington, MA).

In an additional series of experiments, venous blood was similarly withdrawn from the right ventricle at the end of the experiment. The mice were spontaneously breathing 100% F_{IO_2}, which resulted in an arterial oxygen saturation of greater than 0.96 in all groups. This mixed venous oxygen saturation reflects the balance between whole body oxygen consumption and whole body oxygen delivery.

Data Analysis

Raw data were tested using a Kolmogorov-Smirnov test and found not to differ significantly from a normal distribution. Therefore, analysis of variance was used to test for differences between groups in measured variables, with $P < 0.05$ indicating significance. When significant, we identified specific differences between groups using sequentially rejective Bonferroni tests for multiple comparisons. Data are expressed as mean ± SD throughout the text, table, and figures.

Results

Interleukin-6 expression in the lungs increased at 6 h after endotoxin intratracheal instillation in the endotoxin control groups ($P < 0.001$, fig. 1). Consistent with this, endotoxin intratracheal instillation resulted in a marked increase in interleukin-6 mRNA expression in the endotoxin control group ($P < 0.05$, fig. 2). Compared with the endotoxin-induced increase observed in endotoxin controls (lipopolysacharide/control in fig. 1), dobutamine decreased interleukin-6 expression by 45% ($P < 0.05$) and decreased interleukin-6 mRNA by 53% ($P < 0.05$, fig. 2). Compared with endotoxin controls, dopexamine infusion in endotoxic mice decreased interleukin-6 protein expression by 29% (P = nonsignificant, fig. 1) and decreased interleukin-6 mRNA by 54% ($P < 0.05$, fig. 2). In further control experiments not receiving endotoxin intratracheally, dobutamine and dopexamine did not alter interleukin-6 protein or mRNA expression, or any of the experimental measures, compared with the PBS–control group.

To determine whether reduced interleukin-6 protein and mRNA expression after dobutamine infusion is caused by increased antiinflammatory cytokine expression, we measured interleukin-10 concentration in the lungs. Similar to previous observations in other models

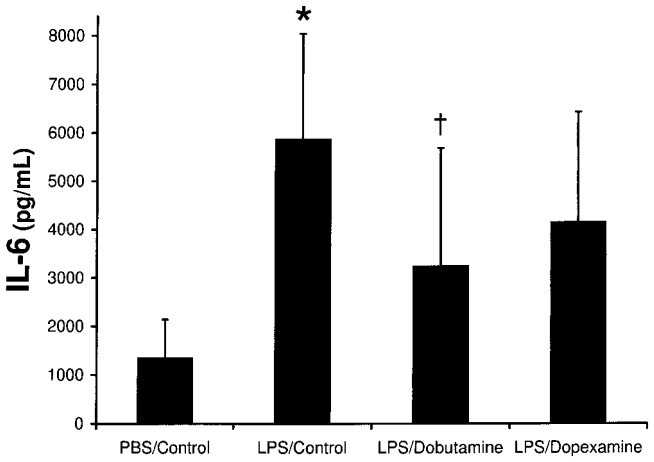

Fig. 1. The mean concentration of interleukin-6 (IL-6) in lung homogenates, measured using enzyme-linked immunosorbent assay, is shown (error bar is SD). Compared with phosphate-buffered saline (PBS)/control (n = 6), intratracheal endotoxin (lipopolysacharide [LPS]/control, n = 12) increased lung interleukin-6 concentration (*$P < 0.05$). Dobutamine infusion decreased this effect of endotoxin (LPS/Dobutamine, n = 13, †$P < 0.05$), whereas dopexamine had a lesser effect (LPS/Dopexamine, n = 13, P = nonsignificant).

of sepsis,[22] pulmonary interleukin-10 is constitutively expressed in the control group and does not significantly increase after endotoxin administration (fig. 3). However, dobutamine infusion decreased pulmonary interleukin-10 expression by 70% ($P < 0.05$), whereas dopexamine infusion had no significant effect (fig. 3). Thus, increased interleukin-10 cannot account for dobutamine-induced decreases in interleukin-6 mRNA and protein expression after endotoxin administration.

Early proinflammatory cytokines cause induction of chemokines. To test whether β-adrenergic agonist–in-

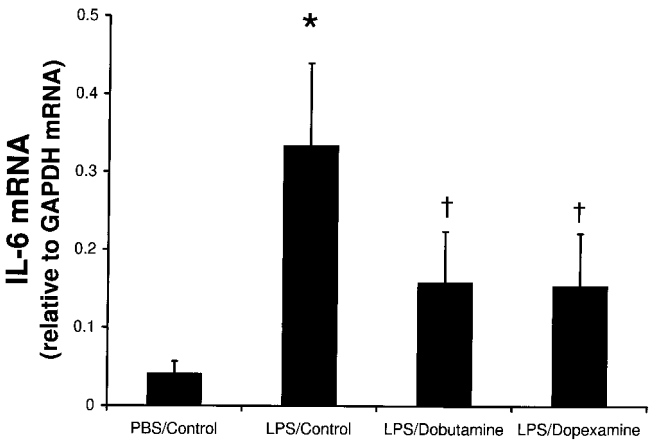

Fig. 2. Mean densitometry of interleukin-6 (IL-6) mRNA (divided by control glyceraldehyde-3-phosphate dehydrogenase [GAPDH] mRNA) in lung extracts, measured using reverse-transcription polymerase chain reaction, is shown (error bar is SD). Compared with control (n = 4), intratracheal endotoxin (n = 6) increased lung IL-6 mRNA expression (*$P < 0.05$). Dobutamine (n = 6) and dopexamine (n = 6) infusion decreased this effect of endotoxin (†$P < 0.05$). PBS = phosphate-buffered saline; LPS = lipopolysaccharide.

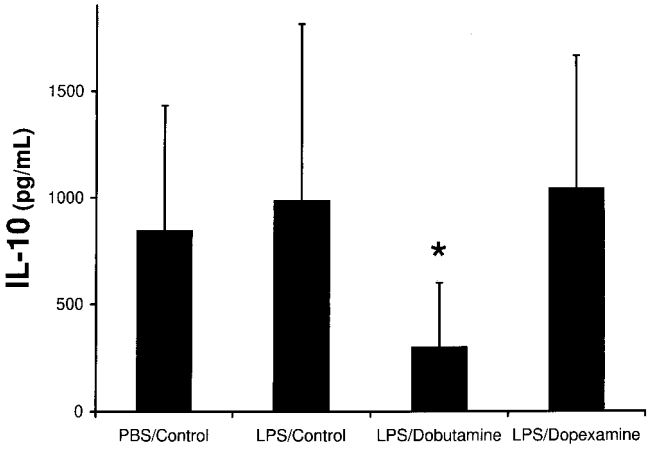

Fig. 3. The mean concentration of interleukin-10 (IL-10) in lung homogenates, measured using enzyme-linked immunosorbent assay, is shown (error bar is SD). Compared with phosphate-buffered saline (PBS)/control (n = 6), intratracheal endotoxin (lipopolysacharide [LPS]/control, n = 12) did not significantly alter pulmonary IL-10 concentration. However, dobutamine infusion significantly decreased pulmonary IL-10 in endotoxemic mice (n = 13, *$P < 0.05$), whereas dopexamine did not (n = 13).

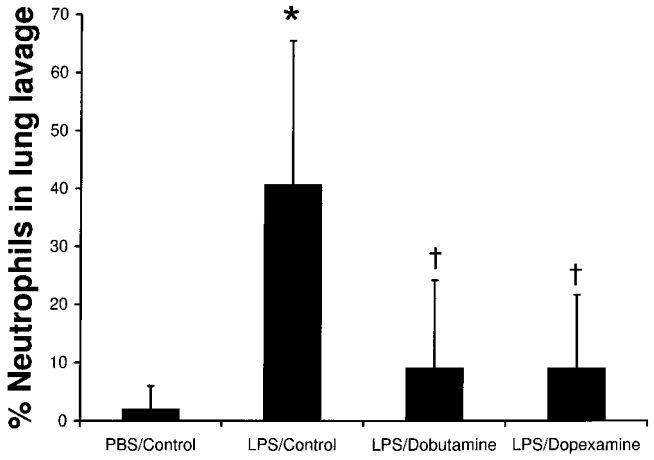

Fig. 5. The mean percent neutrophils measured in cytospins of lung lavage fluid is shown (error bar is SD). Compared with control (n = 4), intratracheal endotoxin (n = 5) resulted in an increase in the percent neutrophils (*$P < 0.05$). Both dobutamine (n = 7) and dopexamine (n = 7) infusions decreased this effect of endotoxin (†$P < 0.05$). PBS = phosphate-buffered saline; LPS = lipopolysaccharide.

duced reductions in interleukin-6 were associated with altered chemokine induction, we measured MIP-2 mRNA expression. Intratracheal endotoxin increased MIP-2 mRNA ($P < 0.05$, fig. 4). Both dobutamine and dopexamine infusion starting immediately before intratracheal endotoxin significantly attenuated the increases in MIP-2 mRNA ($P < 0.05$, fig. 4).

C-X-C chemokines, such as interleukin-8 in humans or MIP-2 in the mouse, are important contributors to increased neutrophil recruitment into the lungs during an inflammatory response. Therefore, we measured the fraction of neutrophils in lung lavage fluid. Intratracheal endotoxin increased the percent neutrophils in fluid (fig. 5). Dobutamine infusion starting immediately before intratracheal endotoxin significantly attenuated this increase by 78% ($P < 0.05$). Dopexamine infusion also resulted in a 78% decrease ($P < 0.05$) in the percent neutrophils in lung lavage fluid compared with endotoxic controls (fig. 5).

Endotoxin infusion significantly increased the wet weight/dry weight ratio of the lungs (table 1). Treatment with dobutamine or dopexamine did not further alter the wet weight/dry weight ratio after endotoxin infusion. Arterial oxygen saturation on room air was 86.8 ± 11.9 in the control group and 78.7 ± 18.8 in the endotoxin-treated groups (P = nonsignificant, table 1). When F_{IO_2} was 1.0, there were no significant differences between groups in mixed venous oxygen saturation.

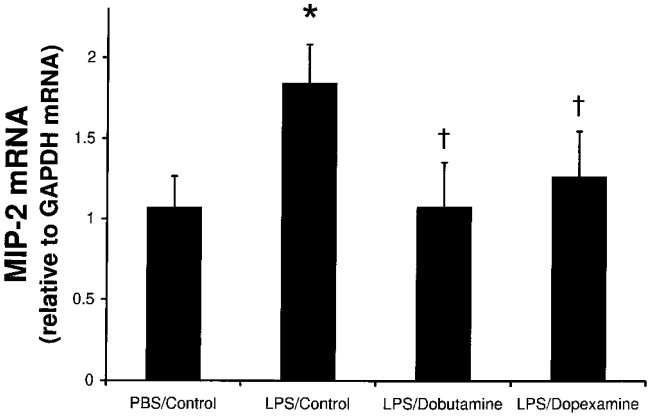

Fig. 4. Mean densitometry of MIP-2 mRNA (divided by control glyceraldehyde-3-phosphate dehydrogenase [GAPDH] mRNA) in lung extracts, measured using reverse-transcription polymerase chain reaction, is shown (error bar is SD). Compared with control (n = 4), intratracheal endotoxin (n = 6) increased lung MIP-2 mRNA expression (*$P < 0.05$). Both dobutamine (n = 6) and dopexamine (n = 6) decreased this effect of endotoxin (†$P < 0.05$). PBS = phosphate-buffered saline; LPS = lipopolysaccharide.

Discussion

This murine model of endotoxin-induced acute lung injury results in a significant increase in early proinflammatory cytokines (interleukin-6) and an associated increase in C-X-C chemokine induction as measured by MIP-2 mRNA expression, with the outcome of increased neutrophils in lung lavage fluid and an increased wet weight/dry weight ratio of the lungs. The key new finding of this study is that dobutamine and dopexamine infusion at clinically relevant doses attenuate these important early steps in the pathogenesis of ARDS. Dopexamine had a lesser effect on interleukin-6 protein expression and did not alter pulmonary interleukin-10 expression. In contrast, dobutamine decreased pulmonary interleukin-10 expression after endotoxin adminis-

Table 1. Lung Wet Weight/Dry Weight Ratio and Oxygen Saturations

	PBS/Control	LPS/Control	LPS/Dobutamine	LPS/Dopexamine
Lung wet weight/dry weight ratio	4.75 ± 0.24 (n = 4)	5.81 ± 1.10* (n = 5)	5.19 ± 0.71 (n = 5)	4.95 ± 1.12 (n = 5)
Arterial O_2 saturation (F_{IO_2} = 0.21)	86.8 ± 11.9 (n = 4)	70.3 ± 29.0 (n = 4)	79.2 ± 18.2 (n = 5)	84.0 ± 11.5 (n = 6)
Venous O_2 saturation (F_{IO_2} = 1.0)	65.5 ± 10.1 (n = 4)	56.6 ± 12.1 (n = 5)	62.0 ± 9.8 (n = 4)	73.1 ± 10.0 (n = 3)

Mean ± SD.

* $P < 0.05$ compared with the PBS/control group.

PBS = intratracheal instillation of phosphate-buffered saline (sham); LPS = intratracheal instillation of lipopolysaccharide; O_2 = oxygen; F_{IO_2} = fraction of inspired oxygen.

tration. These potentially beneficial effects were observed when catecholamine infusion was initiated before lung injury.

These novel findings in the lungs extend previous observations. β-Adrenergic agonists attenuate expression of proinflammatory cytokines (TNF-α and interleukin-6) in cell culture[8,23] and in blood[10,11,24] and attenuate ultrastructural changes of solid organs in animal models of sepsis.[13] Inhibition of β-adrenergic effects using propranolol prevents the effects of adrenaline-mediated suppression of TNF-α.[11] The mechanism of this effect appears to be β_2-adrenergic receptor mediated[23] and involves cyclic adenosine monophosphate.[25] β_2-Receptor binding activates trimeric G proteins, forming stimulatory G proteins.[13] Stimulatory G proteins then activate adenyl cyclase, leading to an increase in intracellular cyclic adenosine monophosphate, which subsequently results in formation of protein kinase A. Protein kinase A inhibits both phospholipase C β and mitogen-activated protein kinase. Phospholipase C β, *via* diacylglycerol and by increasing intracellular calcium through inositol triphosphate, causes an increase in gene transcription for inflammatory proteins (cytokines, integrins, and selectins) and inflammatory lipids (leukotrienes, thromboxane, and platelet-activating factor). β-Adrenergic agonists seem to affect both the transcription and translation of interleukin production. For example, Nakamura *et al.*[26] found that modulation by β-adrenergic agonists of interleukin-6 and TNF-α gene transcription and accumulation of mRNA corresponded to altered promoter activity. Jaffe *et al.*[27] have reported inhibition of interleukin-5 transcription in lung fragments treated with isoproterenol. Other investigators have found that β-adrenergic agonists block inflammatory cytokine production at the posttranscriptional level.[9] β-Adrenergic agonists may prevent accumulation of mRNA within microglial cells in culture stimulated by endotoxin.[28]

In contrast, the effect of α-adrenergic receptor stimulation is less clear. Some findings suggest that α-adrenergic receptor stimulation increases proinflammatory cytokine responses.[29–31] Alternatively, van der Poll *et al.*[32] suggested attenuation of inflammation by α-adrenergic agonists.

Much less is known about the effect of β-adrenergic agonists in the lung. We found that dobutamine and dopexamine inhibited pulmonary interleukin-6 protein and mRNA expression in our model of acute lung injury. Dobutamine decreased pulmonary interleukin-10 expression, whereas dopexamine did not. However, both dobutamine and dopexamine decreased endotoxin-induced MIP-2 message and pulmonary leukocyte infiltration. Dobutamine and dopexamine differ primarily in their α_1-agonist properties. Dobutamine exists as two enantiomers, a positive (+) and a negative (−). The racemic mixture of dobutamine used clinically has β_1-agonist, β_2-agonist, and α_1-agonist properties. Dopexamine, on the other hand, is primarily a β_2 agonist with dopamine receptor-1 and -2 properties. We are uncertain whether the difference between dobutamine and dopexamine is the result of a difference in specific receptor affinity or a difference in effective dose for a specific receptor. It is interesting to note that other mixed α- and β-adrenergic agonists, epinephrine and norepinephrine, attenuate TNF-α production in both *in vitro* and *in vivo* models of sepsis.[32] Our results appear to be consistent with the observations of Spengler *et al.*[31] and van der Poll *et al.*,[32] which suggest that mixed α- and β-adrenergic agonist–like dobutamine may have somewhat different antiinflammatory effects compared with agonists such as dopexamine, which lack α-adrenergic agonist activity. Our observations of differences in interleukin-6– and interleukin-10–modulating effects of dobutamine and dopexamine highlight the results of Tighe *et al.*,[13] who, in a series of experiments with porcine peritonitis, found protective effects from dopexamine and a worsening of hepatic ultrastructural changes with dobutamine. Whether these two adrenergic agents have differential effects on proinflammatory and antiinflammatory cytokines in the liver, as we have observed in the lung, has not yet been described.

Interestingly, we observed a significant decrease in pulmonary interleukin-10 expression after endotoxin exposure in the dobutamine group. Accordingly, dobutamine's effect of decreasing pulmonary interleukin-6 expression could not have been caused by regulation by interleukin-10. In contrast to our results, Suberville *et*

al.[33] found that isoproterenol increased interleukin-10 mRNA and protein expression by macrophages, and van der Poll *et al.*[32] found that epinephrine infusion during human endotoxemia increased circulating interleukin-10 concentrations. Our results in the lung may differ from these previously reported effects of adrenergic agonists on interleukin-10 production for several possible reasons. First, regulation of pulmonary interleukin-10 expression is different from blood interleukin-10 expression. Interleukin-10 is normally expressed in high concentrations in the lungs and does not increase substantially even in models of severe sepsis,[22] even though blood interleukin-10 concentrations and interleukin-10 concentrations in cell culture systems increase dramatically. Thus, previous studies of blood levels and production by isolated cell cultures may differ. Second, changes in specific cytokine expression after endotoxin stimulation is differentially regulated,[34,35] and, in particular, increases in interleukin-10 in the blood after endotoxin challenge appears to depend on combined α- and β-adrenergic stimulation.[32] Furthermore, time of exposure to catecholamines alters outcome. For example, isoproterenol-induced increases in interleukin-10 release from endotoxin-activated mouse peritoneal macrophages occurred only if the mouse was exposed to the β-agonist for at least 2 h before the endotoxin challenge.[33] Similarly, altered interleukin-10 blood concentrations in humans depended on the time of exposure to catecholamine.[32] Thus, the effect of catecholamines on interleukin-10 expression, and interleukin-10 expression in the lungs in particular, may be complex and depend on multiple competing influences.

Conceivably, hemodynamic differences induced by endotoxin, dobutamine, and dopexamine may also contribute to differences in the pulmonary inflammatory response. We chose to measure mixed venous oxygen saturation while F_{IO_2} was 1.0 (which resulted in oxygen saturation > 0.98; table 1). This provides an estimate of the balance between whole body oxygen consumption and oxygen delivery. Oxygen delivery is directly related to cardiac output since oxygen saturation was high and constant and hemoglobin was almost certainly constant. The observation that there were no significant differences in mixed venous oxygen saturation between the groups suggests that cardiac output differences between the groups may not be a major contributor to differences in the pulmonary inflammatory response. However, mixed venous oxygen saturation as a surrogate measure of cardiac output has significant limitations so that the possibility that changes in systemic and pulmonary hemodynamics may have contributed to differences in the pulmonary inflammatory response must be acknowledged.

In summary, in a murine model of acute lung injury, dobutamine and dopexamine inhibit the pulmonary inflammatory response consisting of early proinflammatory cytokine expression, induction of chemokines, and infiltration of the lung with neutrophils. Dopexamine had a lesser effect on interleukin-6 protein expression and did not alter pulmonary interleukin-10 concentration, whereas dobutamine decreased pulmonary interleukin-10 expression. These results suggest that clinically used β-adrenergic agonists may have an effect on the pulmonary inflammatory response in human acute lung injury. However, it is important to recognize that the current effects occurred when adrenergic agents were given before endotoxin administration. Therefore, further investigations are necessary to determine whether similar salutory effects are observed when adrenergic agents are initiated after onset of lung injury.

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