Comparative effects of $\alpha$-receptor stimulation and nitrergic inhibition on bronchovascular tone

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Carvalho, Paula, William H. Thompson, and Nirmal B. Charan. Comparative effects of $\alpha$-receptor stimulation and nitrergic inhibition on bronchovascular tone. J Appl Physiol 88: 1685–1689, 2000.—Adrenergic agonists are known to influence bronchial blood flow and bronchovascular resistance. Recently, the nitrergic system has also been implicated in the control of bronchovascular tone. In this study, we compared the effects of the nitric oxide synthase inhibitor N$^\text{-}$nitro-arginine methyl ester (L-NAME) and the $\alpha_1$-receptor agonist phenylephrine on bronchovascular resistance in anesthetized sheep ($n = 9$). Bronchial blood flow, cardiac output, and systemic and pulmonary arterial pressures were continuously monitored. Phenylephrine (1.2–3.4 $\mu$g·kg$^{-1}$·min$^{-1}$) was infused intravenously to increase mean systemic arterial pressure above 95 Torr for 10 min and then was discontinued. When hemodynamic parameters returned to baseline, nebulized phenylephrine (10 mg) was given over 10 min. When parameters again normalized, L-NAME (30 mg/kg) was infused intravenously over 1 min. Intravenous phenylephrine decreased systemic vascular resistance by 40% at 10 min while no concurrent increase in bronchovascular resistance, but intravenous phenylephrine increased bronchovascular resistance by 66% at 10 min. Thus, comparison intravenous L-NAME produced a rapid and sustained five-fold increase in bronchovascular resistance at 10 min. We conclude that, although $\alpha_1$-agonist stimulation has some influence on bronchovascular resistance in sheep, the nitrergic system has predominant control of bronchovascular tone.

bronchial circulation; nitric oxide synthase; phenylephrine; $\alpha_1$-adrenoceptor agonists; $\alpha_1$-receptor agonists

The regulation of bronchovascular tone has generated considerable interest in recent years. Several investigators have shown that exogenous administration of $\alpha_1$- and $\beta$-adrenoceptor agonists influences bronchial blood flow and bronchovascular tone (1, 2, 4–9). The sympathetic nervous system was previously thought to be the main regulator of the bronchial circulation; however, the nitrergic system has more recently also been implicated in the control of bronchial blood flow. We have recently studied the relationship of $\beta$-adrenoceptors and nitric oxide in the bronchial circulation and found that $\beta$-agonists cause bronchial vascular relaxation, which is partially mediated through the synthesis of endogenous nitric oxide (2). Additionally, we found that $\beta$-agonists and nitric oxide have a synergistic vasodilatory effect on bronchial blood flow (4). However, the comparative roles of $\alpha_1$-adrenoceptor agonists and the nitrergic system in the control of bronchial vascular tone are not known. Therefore, to determine the relative contributions of nitric oxide and $\alpha_1$-adrenoceptor agonists in the regulation of bronchial blood flow, we compared the effects of the $\alpha_1$-adrenoceptor agonist phenylephrine and nitric oxide synthase (NOS) inhibition with N$^\text{-}$nitro-arginine methyl ester (L-NAME) on bronchovascular tone in an anesthetized sheep model.

The bronchial circulation is a unique vascular system because pharmacological agents can be administered directly by intravascular injection or by inhaled aerosol (1–4, 11). Thus agents administered by the intravascular route first act on the endothelium to rapidly alter vascular smooth muscle function through the nitrergic system. In contrast, agents administered by inhalation are initially deposited on the airway epithelium, are then absorbed through the mucosa, and subsequently penetrate the vascular wall to reach the vascular endothelium. These agents may then first act directly on the vascular smooth muscle before reaching the vascular endothelium. The route of drug delivery into the bronchial vascular bed may therefore elicit differential responses in bronchial blood flow. For this reason, in this study, phenylephrine was administered by systemic infusion as well as by inhaled aerosol to determine whether the mode of delivery produced differential effects on bronchovascular tone.

METHODS

Surgical Preparation

Nine adult sheep of mixed breeds (body weight 70–80 kg) were fasted for 12 h and sedated with xylazine (0.5 mg/kg). After induction of anesthesia with thiamylal sodium (15–20 mg/kg iv), the animals were intubated and anesthesia was maintained with 1–2% halothane. Supplemental O$_2$ was provided to maintain the arterial P$_{O2}$ above 100 Torr to prevent hypoxic vasoconstriction and hypoxemia-induced changes in bronchial blood flow. The animals were ventilated with a tidal volume of 10 ml/kg and a respiratory rate of 10 breaths/min (Ohmeda Anesthesia System Excel 210, Madison, WI), and these settings were adjusted to maintain the arterial P$_{CO2}$ between 35 and 40 Torr. The rumen was vented with an orogastric tube.

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A left thoracotomy was performed through the fifth intercostal space, the bronchoesophageal trunk was identified, and the common bronchial branch was isolated. A 2-mm ultrasonic flow probe (Transonic Systems, Ithaca, NY) was placed around the common bronchial branch of the bronchoesophageal artery for determination of bronchial blood flow. The pericardium was opened, and a 16-mm flow probe was placed around the pulmonary trunk for determination of cardiac output. After placement, the flow probes were connected to a dual-channel ultrasonic blood flowmeter (model T201, Transonic Systems). The chest was then closed, and the lung was reexpanded. A Silastic catheter for arterial blood sampling and arterial pressure measurement was placed in the left carotid artery, and a balloon-tipped pulmonary artery catheter (Pentalumen 8 Fr, Abbott, N. Chicago, IL) was advanced into the pulmonary artery via the left jugular vein. Heparin (10,000-U bolus) was given to prevent blood clotting of the catheters. An 18-gauge needle was inserted in the endotracheal tube and connected to a calibrated transducer for assessment of airway pressure. Systemic and pulmonary arterial pressures were measured with calibrated transducers (referenced to the left atrium) and continuously recorded on a multichannel system (model 2107-8890-00, Gould, Cleveland, OH). A continuous chart recording of vascular flows as well as hemodynamic pressure parameters was obtained. Arterial blood samples were drawn intermittently under anaerobic conditions from the carotid catheter by using a heparinized syringe and were immediately analyzed for pH, arterial PO$_2$, and arterial PCO$_2$ (model ABL-520, Radiometer, Copenhagen, Denmark).

**Delivery of Agents**

Intravenous phenylephrine. Phenylephrine (1% injection, Neo-Synephrine HCl, Winthrop Pharmaceuticals, New York, NY), 10 mg in 250 ml of 5% dextrose, was given as a continuous intravenous infusion (Calibrated Syringe Pump System 341A, Orion Research, Cambridge, MA) into a peripheral vein.

Phenylephrine by inhalation. A small-volume nebulizer (Salter Laboratories, Arvin, CA) was connected between the endotracheal tube and the ventilator tubing and secured with a T-adapter. Phenylephrine, 10 mg in 2.5 ml of normal saline, was administered via continuous nebulization. The nebulizer was connected to an E cylinder and driven with oxygen at a flow rate of 8 l/min for a total of 10 min/dose.

L-NAME. L-NAME HCl (Sigma Chemical, St. Louis, MO), 30 mg/kg was dissolved in 20 ml of normal saline and administered over 1 min by continuous intravenous infusion into a peripheral vein.

**Experimental Protocol**

We first tested the response in bronchial blood flow to systemic intravenous administration of phenylephrine by infusing the agent via a hindlimb vein. Baseline parameters, including bronchial blood flow, cardiac output, and mean systemic and pulmonary arterial pressures, were obtained. Phenylephrine was infused at an initial rate of 1 ml/min and increased every 2–3 min until mean systemic arterial pressure exceeded 95 Torr. The infusion rate then remained constant (average 2.5 µg·kg$^{-1}$·min$^{-1}$) for 10 min, at which time it was discontinued. Hemodynamic parameters and blood flows were obtained at 5-min intervals. Bronchovascular resistance was calculated with the following equation (5):

$$\text{bronchial blood flow} \times \text{mean pulmonary arterial pressure} - \text{mean systemic arterial pressure}$$

$$\text{cardiac output}$$

Systemic vascular resistance was calculated with the equation:

$$\text{mean systemic arterial pressure} - \text{mean right atrial pressure}$$

$$\text{cardiac output}$$

When all hemodynamic parameters and blood flows had returned to baseline values (~30 min after the infusion was stopped), phenylephrine was then administered via nebulizer over 10 min as described in Delivery of Agents. Data were obtained for an additional 60 min or until hemodynamic parameters and blood flows returned to baseline.

When all parameters returned to baseline, L-NAME (30 mg/kg) was administered intravenously via a peripheral vein as a continuous infusion over 1 min. Hemodynamic parameters and blood flows were then recorded at 5-min intervals for 90 min.

**Statistical Analysis**

Changes in hemodynamic parameters and blood flows over time were compared by means of a one-way analysis of variance followed by Dunnett’s test. The changes between inhaled and intravenous phenylephrine and between intravenous phenylephrine and L-NAME were compared by the paired t-test. A P value of <0.05 was considered significant. All data are presented as means ± SE.

**RESULTS**

All of the animals used in this study were adult sheep (mean weight 75.2 kg, range 70–80 kg) without evidence of cardiac or respiratory system disease.

**Effects of Intravenous Phenylephrine**

Baseline bronchial blood flow was 22 ± 5.8 ml/min. Intravenous phenylephrine at doses sufficient to increase the mean systemic arterial pressure above 95 Torr doubled bronchial blood flow at 10 min (P < 0.05), which then rapidly returned to baseline values when the infusion was discontinued (Fig. 1). Mean systemic arterial pressure increased by ~25% with intravenous phenylephrine and rapidly returned to baseline when the infusion was stopped (P < 0.01; Table 1). Cardiac output was 3.2 ± 0.5 l/min, right atrial pressure was ~5 Torr, and airway pressure was 13 ± 2 Torr at their respective baselines. These parameters did not significantly change with infusion of phenylephrine. Similarly, mean pulmonary arterial pressure was 16 ± 3 Torr at baseline and did not significantly change with intravenous phenylephrine. Bronchovascular resistance increased only slightly from 2.8 to 3.0 Torr$^{-1}$·min$^{-1}$ at 5 min, whereas systemic vascular resistance increased by ~25%. At 10 min, however, bronchovascular resistance decreased by ~30% to 1.9 Torr$^{-1}$·min$^{-1}$ and returned to baseline when the infusion of phenylephrine was discontinued (Fig. 2). Systemic vascular resistance increased by ~40% at 10
min but rapidly decreased with cessation of the infusion (Fig. 2).

Effects of Inhaled Phenylephrine

In contrast to intravenous phenylephrine, inhaled phenylephrine resulted in a decrease in bronchial blood flow from 21.8 ± 6 ml/min at baseline to 15 ± 5 ml/min at 10 min (Fig. 1). Bronchovascular resistance increased by ~66% at 10 min and remained above baseline values for at least an additional 30 min (Fig. 2). As with the intravenous route, there were no significant changes in cardiac output (baseline value of 3.9 ± 0.5 l/min), mean right atrial pressure (~5 Torr), mean pulmonary arterial pressure (baseline value of 15 ± 3 Torr), or airway pressure (baseline value 13 ± 3 Torr) after phenylephrine by inhalation. Additionally, there were no changes in either systemic arterial pressure (Table 1) or systemic vascular resistance with inhaled phenylephrine. Airway pressure did not significantly change with inhaled phenylephrine (mean value 15 ± 2 Torr).

Effects of Intravenous L-NAME

Intravenous injection of L-NAME produced a rapid decrease in bronchial blood flow to ~25% of baseline values (P < 0.001; Fig. 1) with a corresponding fivefold increase in bronchovascular resistance (Fig. 2). These effects were sustained for the remainder of the experiment, at least 90 min. With L-NAME, mean systemic arterial pressure increased by ~28% at 10 min compared with baseline values (P < 0.01; Table 1) with a corresponding 50% increase in systemic vascular resistance (Fig. 2). Mean pulmonary arterial pressure did not significantly change from a baseline of 15 ± 2 Torr, and right atrial pressure remained unchanged at ~5 Torr. There was a nonsignificant decrease in cardiac output with intravenous L-NAME (baseline of 3.5 ± 0.7 to 2.9 ± 0.8 l/min at 10 min).

DISCUSSION

This study demonstrated that the bronchial circulation responds to exogenous administration of the α-receptor agonist phenylephrine, indicating the presence of α-adrenoceptors. We administered phenylephrine by the intravenous route and by aerosol through an endotracheal tube. Interestingly, we found that the effects of phenylephrine on bronchovascular tone differed with the mode of delivery. When phenylephrine was administered by inhalation, it produced an increase in bronchovascular resistance, whereas intravenous administration produced a small initial increase, followed by a subsequent decrease in bronchovascular resistance.

In this study, aerosolized phenylephrine caused an increase in bronchovascular resistance without a change in systemic vascular resistance. This indicates that

<table>
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<th>Treatment</th>
<th>Time, min</th>
<th>Mean Systemic Arterial Pressure, Torr</th>
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<tr>
<td>Phenylephrine (intravenous)</td>
<td>0</td>
<td>74 ± 3</td>
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<tr>
<td></td>
<td>5</td>
<td>100 ± 4*</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>103 ± 6*</td>
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<td></td>
<td>20</td>
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<tr>
<td></td>
<td>5</td>
<td>92 ± 5</td>
</tr>
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<td></td>
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<td>20</td>
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<td>80 ± 6</td>
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Values are means ± SE. L-NAME, N-nitro-L-arginine methyl ester. *P < 0.05 vs. baseline.
aerosolized phenylephrine was able to penetrate the vascular wall and act directly on vascular smooth muscle. However, it must not have crossed the entire vascular wall and, hence, did not enter the systemic circulation. A similar finding was observed in a previous study (3), in which aerosolized acetylcholine, even in large doses, did not cause effects in the peripheral vasculature. In that study, peripheral intravenous injection of acetylcholine (2 µg/kg) produced a decrease in systemic arterial pressure as well as a marked (75%) decrease in bronchovascular resistance. However, when acetylcholine was aerosolized at doses of 2 and 20 µg/kg, bronchovascular resistance actually increased by ~10% without a change in systemic vascular resistance. In contrast, a much larger dose of aerosolized acetylcholine (up to 2,000 µg/kg) produced a 15% decrease in bronchovascular resistance with no change in systemic vascular resistance. These findings suggest that aerosolized acetylcholine readily penetrates the bronchial mucosa and acts directly on the vascular smooth muscle but does not appear to enter the systemic circulation even when larger doses are used. Similarly, in the present study, the dose of phenylephrine given by inhalation (2.5 mg/10 ml NaCl) increased bronchovascular resistance without significant effects on peripheral vascular resistance.

The effect of aerosolized phenylephrine on tracheal mucosal blood flow has been studied by Barker et al. (1) in a sheep model. In their study, tracheal mucosal flow was measured by an inert soluble gas technique using dimethyl ether (15). Barker and colleagues found that phenylephrine administered as an aerosol reduced mucosal blood flow in a dose-dependent manner and that this response was partially blocked by phentolamine (1). In their study, they used concentrations ranging from 0.25 to 2.0 mg of phenylephrine/ml of buffered saline and observed a maximal reduction of 70% decrease in tracheal mucosal flow (normalized for systemic arterial pressure) with the highest dose. In our study, we used a higher dose of phenylephrine (4 mg/ml of buffered saline) and observed a comparatively smaller reduction in bronchial blood flow. These differences are likely a result of the difference in model because mucosal blood flow was measured in a tracheal chamber in the study by Barker et al., whereas total bronchial blood flow was measured directly with an ultrasonic flow probe in our study. The latter technique allows measurement of total blood flow to both the submucosal and peribronchial vascular networks of the airway wall. The effect of phenylephrine was therefore likely to be distributed over a larger vascular area, and we consequently observed comparatively less effect in bronchovascular resistance.

Intravenous injection of phenylephrine produced interesting results: at 5 min after administration of intravenous phenylephrine was started, systemic vascular resistance increased by ~25%, but bronchovascular resistance increased only slightly from 2.8 to 3.0 Torr·ml⁻¹·min⁻¹. Thus intravenous phenylephrine must have resulted in a greater degree of vasoconstriction in the peripheral systemic vascular bed relative to vasoconstriction of the bronchial vasculature, suggesting that the concentration of α-receptors in the bronchial circulation is lower compared with the peripheral arterial circulation. At 10 min after injection, however, bronchovascular resistance decreased by ~30% to 1.9 Torr·ml⁻¹·min⁻¹ despite the absence of further changes in systemic arterial pressure. A possible explanation for this decrease in bronchovascular resistance at 10 min is that phenylephrine-induced vasoconstriction stimulated release of endogenous nitric oxide in the bronchial vasculature. This contention is further supported by the fact that, after aerosolized phenylephrine, there was a trend for mean bronchovascular resistance to eventually decrease following the initial increase. Indeed, it has previously been shown that phenylephrine-induced vasoconstriction may stimulate synthesis of endogenous nitric oxide (13, 14). Thus aerosolized phenylephrine also caused initial bronchial vasoconstriction followed by a reactive hyperemia, which was likely mediated through the release of endogenous nitric oxide.

The effect of α-adrenergic stimulation on bronchial blood flow has also been studied by using closed intraarterial injection of α-adrenoceptor agonists directly into the bronchial artery. Direct injection of epinephrine (5) and phenylephrine (10) has been shown to produce an increase in bronchovascular tone and a decrease in bronchial blood flow. The method of direct injection into the bronchial artery obviates any systemic effects resulting from α-receptor stimulation in the peripheral vascular bed and exposes only the bronchial vasculature to these agents. In contrast, in our study, systemic intravenous administration of phenylephrine resulted in stimulation of all α-adrenoceptors in the systemic vascular bed. Therefore, in view of the predominant systemic effects of intravenous phenylephrine infusion, compared with selective bronchial arterial injection, these findings support the assumption that α-receptors in the bronchial circulation are present in a lower concentration compared with α-receptors in the systemic circulation.

The blood flow in various vascular beds is regulated by different mechanisms. In the past few years, the relative contribution of the nitrergic system in the regulation of blood flow has been intensively studied. For example, Shrier and Magder (12) studied the effects of NOS inhibition and phenylephrine in the dog femoral artery in a model of isolated hindlimb vasculature. These investigators found that NOS inhibition with NG-nitro-L-arginine and phenylephrine had similar hemodynamic effects with both producing an increase in vascular resistance, indicating that the femoral artery has both α-adrenergic and nitricergic control mechanisms. In the bronchial circulation, the factors that regulate blood flow have not yet been clearly defined. Therefore, one purpose of the present study was to determine the relative contributions of nitric oxide and sympathetic α-adrenoceptors in the control of resting bronchovascular tone. To study this, we administered both L-NAME and phenylephrine by systemic intravenous injection to produce approximately the
same mean systemic arterial pressure and, hence, a similar driving pressure for bronchial blood flow. We found that, despite the comparable hemodynamic responses produced by these two agents, intravenous phenylephrine produced a decrease in bronchovascular tone, whereas NOS inhibition resulted in a marked increase in bronchovascular resistance which was sustained for an additional 90 min, confirming that the effect of NOS inhibition has a long duration of action as previously described (2, 4, 16). The responses to NOS inhibition and α-adrenoceptor stimulation are, therefore, different in the bronchial vasculature compared with the femoral artery. The findings in this study suggest that, although the bronchial circulation contains α-adrenoceptors, resting bronchovascular tone is predominantly regulated through the synthesis of endogenous nitric oxide and that, compared with the peripheral vasculature, the bronchial circulation is more dependent on nitrergic control. Thus we conclude that, in sheep, the nitrergic system appears to be the predominant mechanism for regulation of bronchovascular tone.

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