Non-cAMP-mediated bronchial arterial vasodilation in response to inhaled β-agonists

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Carvalho, Paula, Shane R. Johnson, Nirmal B. Charan. Non-cAMP-mediated bronchial arterial vasodilation in response to inhaled β-agonists. J. Appl. Physiol. 84(1): 215–221, 1998.—We studied the dose-dependent effects of inhaled isoetharine HCl, a β-adrenergic bronchodilator (2.5, 5.0, 10.0, and 20.0 mg), on bronchial blood flow (Qbr) in anesthetized sheep. Isoetharine resulted in a dose-dependent increase in Qbr. With a total dose of 17.5 mg, Qbr increased from baseline values of 22 ± 3.4 (SE) to 60 ± 16 ml/min (P < 0.001), an effect independent of changes in cardiac output and systemic arterial pressure. To further study whether synthesis of endogenous nitric oxide (NO) affects β-agonist-induced increases in Qbr, we administered isoetharine (20 mg) by inhalation before and after the NO-synthase inhibitor Nω-nitro-L-arginine methyl ester (L-NAME). Intravenous L-NAME (30 mg/kg) rapidly decreased Qbr by ~80% of baseline, whereas L-NAME via inhalation (10 mg/kg) resulted in a delayed and smaller (~22%) decrease. Pretreatment with L-NAME via both routes of administration attenuated bronchial arterial vasodilation after subsequent challenge with isoetharine. We conclude that isoetharine via inhalation increases Qbr in a dose-dependent manner and that β-agonist-induced relaxation of vascular smooth muscle in the bronchial vasculature is partially mediated via synthesis of NO.

bronchial circulation; β-adrenergoreceptor agonists; nitric oxide; adenosine 3',5'-cyclic monophosphate

DIRECT INFUSION of β-adrenergoreceptor agonists into the bronchial artery in sheep produces marked increases in bronchial blood flow (Qbr), demonstrating that β-receptors are present on the bronchial arteries (22). Similarly, when administered via inhalation, isoproterenol has been found to increase tracheal mucosal blood flow (2). The relaxation of vascular smooth muscle resulting from administration of β-agonists has conventionally been attributed to occur via activation of adenylate cyclase resulting in an increase in adenosine 3',5'-cyclic monophosphate (cAMP) in the vascular smooth muscle cell (13, 16). However, it has recently been shown that this effect is attenuated by removal of the vascular endothelium (24, 26) or by pretreatment with inhibitors of nitric oxide synthase (NOS) (10–12, 23, 26). In view of the potential interaction between the β-agonist and nitric oxide (NO) pathways, we hypothesized that increases in Qbr in response to β-agonists are partially mediated through synthesis of endogenous NO. Therefore, to determine the potential contribution of NO in β-agonist-mediated effects in the bronchial circulation in vivo, we first administered isoetharine HCl via inhalation to adult, anesthetized sheep to establish a dose-response relationship in Qbr. We then tested the effect of NOS inhibition on β-agonist-mediated bronchial arterial vasodilation with a subsequent challenge of isoetharine HCl. The NOS inhibitor, Nω-nitro-L-arginine methyl ester (L-NAME) was administered intravenously or by inhalation to establish whether it produced similar effects on β-agonist-induced alterations in Qbr with these two different modes of administration. A sheep model was chosen for this study because of similarities in the tracheobronchial circulation to those in the human (19) and because the sheep has a dense submucosal bronchial vascular plexus (5).

METHODS

Surgical Preparation

Adult sheep of mixed breeds (70–80 kg body wt) were fasted for 12 h and then sedated with xylazine (0.5 mg/kg im). After induction of anesthesia with thiymal sodium (15–20 mg/kg iv), the animals were intubated and anesthesia was maintained with 1–2% halothane. Supplemental O2 was provided to maintain the arterial PO2 (PaO2) well above 100 Torr to minimize hypoxic vasoconstriction and hypoxemia-induced changes in Qbr. 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 PCO2 (PaCO2) between 35 and 40 Torr. The rumen was vented with an orogastric tube.

A left thoracotomy was performed through the fifth intercostal space, the bronchoesophageal trunk was identified, and the common bronchial branch was isolated. An ultrasonic flow probe (2 mm, 2RS, Transonic Systems, Ithaca, NY) was placed around the common bronchial branch of the bronchoesophageal artery for determination of Qbr. Caution was taken to avoid compression or torsion of the vessel. The pericardium was then opened, and a second flow probe (16 mm, 16SS) was placed around the pulmonary trunk for determination of cardiac output (Qt). After insertion, the flow probes were tested by connecting them successively to an ultrasonic blood flowmeter (Transonic Systems T201) to ensure that a strong pulsatile reading was being obtained. 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. A balloon-tipped pulmonary artery catheter (Pentalumen Thermodilution 8 Fr, Abbott, N. Chicago, IL) was placed via the left jugular vein and advanced into the pulmonary artery. An 18-gauge needle was inserted in the endotracheal tube and connected to a calibrated transducer for assessment of airway pressure. Systemic arterial pressure (Psa) and pulmonary arterial pressure (Ppa) were measured with calibrated transducers (referenced to left atrium) and continuously recorded (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 under anaerobic conditions from the carotid catheter.
using a heparinized syringe and were analyzed for pH, P\textsubscript{A\textsubscript{O\textsubscript{2}}}, and P\textsubscript{A\textsubscript{CO\textsubscript{2}}}, (Radiometer ABL-520, Copenhagen, Denmark).

**Delivery of Inhaled Agents**

Isoetharine HCl. A small-volume nebulizer (Salter Labs, Arvin, CA) was connected between the endotracheal tube and the ventilator tubing and secured with a T adapter. Isoetharine HCl inhalation solution (USP, 1%, Winthrop Pharmaceuticals, New York, NY) was placed in the nebulizer in graduated doses (2.5, 5.0, 10.0, and 20.0 mg) and diluted with sterile NaCl (0.9%) to achieve a total volume of 2.5 ml with each dose, thus yielding a final concentration range of 3.6 × 10\textsuperscript{-3} to 2.9 × 10\textsuperscript{-4} M. The nebulizer was connected to an O\textsubscript{2} cylinder and driven with O\textsubscript{2} at a flow rate of 8 l/min for a total of 8 min/dose, the time required to empty the nebulizer.

L-NAME. L-NAME HCl (Sigma Chemical, St. Louis, MO), 10 mg/kg, was dissolved in 2.5 ml of sterile 0.9% NaCl. We were unable to use higher amounts because, at this dose, we had achieved a saturated solution. Therefore, at higher doses, the physical properties of the mixture impaired adequate delivery. L-NAME was administered via Salter nebulizer driven by O\textsubscript{2} at a flow rate of 8 l/min for 10 min.

Phenylephrine. Phenylephrine HCl (1% injection; Neo-Synephrine, Winthrop Pharmaceuticals, New York, NY), 10 mg in 2.5 ml of normal saline, was administered via continuous nebulization (Salter) driven by O\textsubscript{2} at a flow rate of 8 l/min for 10 min.

NO. A premixed cylinder of NO (Airco, BOC Group, Murray Hill, NJ) containing NO, 600 parts per million (ppm) in N\textsubscript{2}, was connected to the nitrous oxide port of the anesthesia machine via a stainless steel regulator. The NO-N\textsubscript{2} mixture was blended with O\textsubscript{2} and air to achieve an approximate concentration of 100 ppm. Although the exact concentration of NO was not measured, each dose administered in this study was uniform.

**Experimental Protocol**

**Experiment 1.** Dose-response relationship of aerosolized isoetharine HCl on Q\textsubscript{br} (n = 6). Baseline physiological parameters, including Q\textsubscript{br}, Q\textsubscript{T}, P\textsubscript{sa}, end-inspiratory airway pressure (Paw), P\textsubscript{pa}, and arterial blood gases, were obtained. Bronchial vascular resistance (BVR) was calculated using the equation (6): BVR = P\textsubscript{sa} - P\textsubscript{pa}/Q\textsubscript{br} (Torr·min·ml\textsuperscript{-1}), in which the mean values of P\textsubscript{sa}, P\textsubscript{pa}, and Q\textsubscript{br} were used for each data point.

A small-volume nebulizer was connected to the endotracheal tube via a T adapter. A control dose of sterile 0.9% NaCl (2.5 ml) was administered by nebulization over 8 min (O\textsubscript{2} flow of 8 l/min), and hemodynamic parameters were obtained for 20 min after completion of treatment. Isoetharine HCl was then nebulized in progressively increasing doses as previously described over 8 min. Arterial blood gases and hemodynamic parameters were obtained before and after each dose. Hemodynamic parameters were obtained at 5-min intervals for 20 min after completion of each dose or until blood flows and hemodynamic parameters had stabilized.

**Experiment 2.** Effect of NO on isoetharine HCl-induced bronchial arterial vasodilation (n = 4). In four of the above animals, NO challenge was given before and after the isoetharine dose-response protocol. Because β-agonists are thought to act predominantly via the production of cAMP (13,16) and NO acts via guanosine 3',5'-cyclic monophosphate (cGMP) (20), the purpose of this protocol was to investigate whether NO produces additional vasodilatory effects after maximal vasodilation has already been achieved with β-agonists. NO was administered at 100 ppm via inhalation for 5 min before beginning the treatment protocol with nebulized isoetharine HCl. Physiological parameters and arterial blood gases were obtained before and after treatment with NO. After NO was obtained, a stabilization period of 20 min was allowed before administration of the first dose of isoetharine HCl. Twenty minutes after the last dose of isoetharine was given, NO (100 ppm) was again administered for 5 min. Arterial blood gases and hemodynamic parameters were then obtained before and after administration of NO.

**Experiment 3.** Effect of NOS blockade on isoetharine HCl-induced alterations in Q\textsubscript{br} (n = 10). In six animals, a single dose of isoetharine HCl was administered via nebulizer (20.0 mg, nebulizer volume 2.5 ml), and parameters were obtained during the 20-min stabilization period as before. L-NAME (30 mg/kg in 20 ml of NaCl) was then administered intravenously over 1 min via a peripheral vein (4), and parameters were observed for a 20-min stabilization period in three animals and for 80 min in three other animals. After the stabilization period, a subsequent challenge with nebulized isoetharine HCl (20.0 mg, nebulizer volume 2.5 ml) was then given as before, and parameters were obtained until stable.

To compare the effects of L-NAME administered intravenously with those of L-NAME administered by inhalation, the latter was given to four separate sheep. In two of these animals, a control dose of isoetharine HCl (20.0 mg, nebulizer volume 2.5 ml) was administered over 8 min, and parameters were obtained for 20 min as before. To determine whether prior β-agonist administration influences the response to a second challenge, a control dose of nebulized 0.9% NaCl (2.5 ml) was administered over 8 min, and parameters were obtained for 20 min in the other two sheep. In all four animals, L-NAME (10 mg/kg in 2.5 ml of NaCl) was then administered via nebulizer over 10 min. Parameters were observed until stable, a period of time that ranged from 60 to 80 min after nebulized L-NAME. At this point, a second dose of isoetharine HCl (20.0 mg, nebulizer volume 2.5 ml) was administered via inhalation to all four animals, and parameters were again observed until stable.

**Experiment 4.** Response to isoetharine HCl after pretreatment with phenylephrine (n = 7). To further determine whether the inhibitory effect of NOS on isoetharine-induced bronchial arterial vasodilation depends on the increased vascular tone, additional animals were treated with phenylephrine as a bronchial arterial vasoconstrictor. Three animals were given phenylephrine by inhalation (10 mg in 2.5 ml of NaCl over 10 min) to determine the extent and duration of the effect on Q\textsubscript{br} and BVR. Hemodynamic parameters and blood flows were obtained at frequent intervals until they returned to baseline, an additional 30–80 min. Four separate animals were then treated with nebulized phenylephrine for 10 min as before, immediately followed by nebulized isoetharine (20 mg over 8 min), and parameters were observed until stable.

**Statistical Analysis**

For the isoetharine HCl dose-response studies, the changes in hemodynamic parameters and arterial blood gases over time were compared by means of a one-way analysis of variance followed by Dunnett’s test. The changes in Q\textsubscript{br} and other hemodynamic parameters before and after treatment with L-NAME were compared with the respective baseline values in each group by the paired t-test. P \textless 0.05 was considered significant. All data are presented as means ± SE.

**RESULTS**

**Experiment 1**

Baseline bronchial blood flow was 22 ± 3.4 ml/min, and there were no significant changes in Q\textsubscript{br} with the
were no further significant increases in Q˙br with 20 mg P, fold increase over baseline values (ing doses of isoetharine HCl (Table 1). With a control dose of nebulized 0.9% NaCl. With increasing doses of nebulized isoetharine HCl, there was a progressive and dose-related increase in Qbr (Fig. 1A). With a nebulized dose of 10 mg, Qbr reached a plateau of ~60 ± 16 ml/min, representing an approximate three-fold increase over baseline values (P < 0.001). There were no further significant increases in Qbr with 20 mg of isoetharine HCl.

There were no dose-related effects in Q˙T with increasing doses of isoetharine HCl (Table 1). With a control dose of nebulized 0.9% NaCl or increasing doses of isoetharine HCl, there were no changes in Psa, Ppa, PAO2, or Paw (Table 1). PAO2 increased from 38 ± 1 Torr immediately before isoetharine to 42 ± 2 Torr at the end of the dose-response study (P < 0.001) after the 10-mg dose, and did not change with the subsequent 20-mg dose (Fig. 1B).

Experiment 2

In four of the animals described above, NO at 100 ppm was delivered via inhalation before the isoetharine dose-response relationship study was conducted. With NO, Qbr increased by ~50% of pre-NO baseline values, from a baseline of 20 ± 2 to a peak of 30 ± 5 ml/min at 5 min (P < 0.01). When NO was discontinued at 5 min, Qbr rapidly returned to baseline (Fig. 2). After administration of the final dose of isoetharine and after a stabilization period of at least 20 min, NO was again given via inhalation at 100 ppm. Administration of NO after isoetharine HCl resulted in an additional increase in Qbr from 53 ± 9 to a peak of 64 ± 10 ml/min, which represents a proportionally smaller increase in Qbr.

Table 1. Effect of inhaled isoetharine on Q˙T, Psa, Ppa, Paw, arterial PO2, and arterial PCO2

<table>
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<th>Time, min</th>
<th>Q˙T, l/min</th>
<th>Psa, Torr</th>
<th>Ppa, Torr</th>
<th>Paw, Torr</th>
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<td>0</td>
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<td>20</td>
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<td>16 ± 2</td>
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<td>38 ± 1</td>
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<td>87 ± 4</td>
<td>15 ± 1</td>
<td>18 ± 1</td>
<td>235 ± 32</td>
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<tr>
<td>20</td>
<td>2.8 ± 0.3</td>
<td>88 ± 4</td>
<td>16 ± 1</td>
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<td>19 ± 1</td>
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<td>42 ± 2*</td>
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<tr>
<td>20</td>
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<td>85 ± 3</td>
<td>16 ± 2</td>
<td>19 ± 0.5</td>
<td>219 ± 23</td>
<td>42 ± 2*</td>
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Values are means ± SE. NaCl, saline; Iso, isoetharine HCl; Q˙T, cardiac output; Psa, mean systemic arterial pressure; Ppa, mean pulmonary arterial pressure; Paw, end-inspiratory airway pressure; PAO2, arterial PO2 and PAO2, respectively. *P < 0.05 compared with initial values.
(−17%) over pre-NO baseline values (P < 0.01). There were no significant changes in QT, Ppa, Psa, Paw, or arterial blood gases with each administration of NO. Also, there were no significant changes in arterial blood gases, vascular flows, or other hemodynamic parameters in these four animals compared with the two animals that were not pretreated with NO for 5 min before the isoetharine HCl dose-response study.

Experiment 3

As shown in Fig. 3, nebulized NaCl produced no significant change in Qbr (23 ± 4 to 20 ± 6 ml/min at 20 min, reflecting a change in BVR from −3.0 to 3.4 Torr·min·ml−1). The first dose of inhaled isoetharine HCl (20.0 mg, nebulizer volume 2.5 ml) produced a greater than twofold rise in Qbr (P < 0.001) and a corresponding decrease in BVR by >50% (P < 0.001). Intravenous l-NAME (n = 6) resulted in a rapid decrease in Qbr of −80% from baseline flow (P < 0.001; Fig. 4A) and a corresponding increase in BVR from 1.5 to 8.0 Torr·min·ml−1 (P < 0.001). Immediately after intravenous injection of l-NAME in all six animals, we observed that there was a sudden and transient increase in Qbr of −25% of baseline values (Fig. 4A). This rapid increase in Qbr peaked within 60 s after completion of the infusion and was temporally associated with a decrease in mean Psa by 10 ± 3 Torr, which was maximal at 60–90 s (Fig. 4B). After this initial increase, Qbr dropped precipitously and reached a nadir −5 min after the infusion of l-NAME. At 3–4 min postinfusion, Psa rose −20 Torr above baseline and remained elevated for the next few minutes, associated with the sustained nadir in Qbr. Mean Ppa began to increase by 5 ± 3 Torr at 3–4 min after infusion (NS), but this effect was transient and no longer present at 20 min (Fig. 4B). QT began to decrease −1–2 min after infusion of l-NAME and reached a nadir of −1.0 l/min at 6–8 min, after which it again began to rise to approximate baseline values. The decrease in Qbr was sustained for the duration of the experiment, at least 80 min. After a second challenge with isoetharine HCl after l-NAME, Qbr showed only a small increase from 10 ± 2 to 14 ± 1 ml/min (Fig. 3), reflecting a change in BVR from −6.9 (immediately before isoetharine) to 5.0 Torr·min·ml−1. There were no significant changes over baseline values in QT, Psa, Ppa, and Paw 20 min after infusion of l-NAME (Table 2). The response in Qbr after this second dose of isoetharine was the same in the three animals that underwent an 80-min stabilization period after intravenous l-NAME compared with the three animals that were allowed to stabilize for only 20 min after l-NAME. There was no significant change in PAO2 or PACO2 with isoetharine or l-NAME.

In contrast to l-NAME administered via the intravenous route, l-NAME delivered via inhalation (n = 4)
resulted in a much smaller and delayed decrease in Qbr of 22 ± 5% from baseline values. The decrease in Qbr with inhaled L-NAME was only evident at around 60–80 min after initial administration. In the two animals pretreated with nebulized NaCl, Qbr increased by 6 ± 3% over baseline at 20 min. With the subsequent isetharine challenge after treatment with nebulized L-NAME in these two animals, Qbr increased by 30 ± 4% over baseline. In the two sheep that were pretreated with nebulized isetharine HCl, Qbr increased by 105 ± 25% over baseline values (P < 0.001). After treatment with nebulized L-NAME, however, the response in Qbr was attenuated, only increasing by 25 ± 5% with the second isetharine challenge (P < 0.001). There were no significant differences in arterial blood gases, Qt, Psa, Ppa, and Paw after treatment with nebulized L-NAME.

Experiment 4

Three animals received phenylephrine (10 mg) nebulized over 10 min. Qbr decreased from 17 ± 3 to 11.6 ± 2 ml/min (P < 0.05), and BVR increased from 4.2 to 6.7 Torr · min · ml⁻¹ 10 min after the start of the treatment. At 20 min, Qbr decreased further to 10.5 ± 1.6 ml/min (P < 0.05; BVR 7.0 Torr · min · ml⁻¹), and bronchovascular tone remained elevated for at least 30 min after phenylephrine nebulization was completed. In four separate animals, phenylephrine was nebulized for 10 min, which resulted in a comparable decrease in Qbr from 18.0 ± 3.9 to 11 ± 1.8 ml/min (P < 0.05) and an increase in BVR from 3.6 to 6.6 Torr · min · ml⁻¹ at 10 min. With administration of nebulized isetharine HCl (20 mg) immediately after phenylephrine, Qbr increased to 29 ± 5 ml/min (P < 0.01; BVR 2.2 Torr · min · ml⁻¹) 20 min after the start of isetharine. This effect persisted for an additional 20 min after completion of treatment.

**DISCUSSION**

The systemic circulation in the lung is known to contain β-adrenergoreceptors (1, 3). Parsons et al. (22) have shown that direct injection of isoproterenol into the bronchial artery in sheep induces an increase in Qbr. Similarly, it has also been shown that administration of β-agonists in the tracheobronchial circulation results in decreased tracheal vascular resistance (17) and increased tracheal mucosal blood flow (2, 7). In this study, we have also found that isetharine, a β-adrenergic agonist, increases Qbr, thus confirming the presence of β-receptors in the bronchial vasculature of sheep (Fig. 1A).

To simulate the clinical condition, we administered a β-adrenergic agonist by inhalation to intact animals to determine the local vascular effects in the bronchial circulation. We found that Qbr markedly increased with the administration of inhaled isetharine HCl, an effect that appeared to be independent of changes in hemodynamic parameters such as Qt, Psa, or Ppa. Additionally, when isetharine was given as a single 20-mg dose, there were no changes in arterial blood gases. We also found that the increases in total Qbr were dose related. These findings are in agreement with those of Barker and colleagues (2), who demonstrated that local exposure of the tracheal mucosa to inhaled isoproterenol resulted in a dose-dependent increase in tracheal mucosal blood flow. However, Wanner et al. (27) measured only the tracheal mucosal blood flow by the inert soluble gas technique using dimethyl ether, whereas we measured the total bronchial arterial blood flow directly with an ultrasonic flow probe, a technique that measures the total blood flow to the airway mucosa as well as that supplying the entire airway wall. Qbr progressively increased with higher doses of isetharine HCl and ultimately reached a plateau. With administration of the last dose of isetharine HCl (20 mg), there were no further significant changes in Qbr or BVR (Fig. 1, A and B), thereby suggesting that maximal β-receptor stimulation had been achieved. However, when exogenous NO was then delivered via inhalation, an additional increase in Qbr was seen, suggesting that exogenous NO produced further vasodilation (Fig. 2). This is not surprising, because NO causes vasodilation through stimulation of soluble guanylate cyclase and production of cGMP (20). It is also possible that prolongation of the half-life of cGMP by cAMP may potentially have contributed to this additional vasodilation (18, 25). However, the increase in Qbr with NO after the last dose of isetharine HCl was proportionally smaller than the increase in Qbr we observed before pretreatment with isetharine. A possible explanation for these findings could be that the near-maximal capacity for bronchial vascular engorgement had been achieved after β-agonist treatment, thus further NO-induced increases in Qbr were less pronounced.

Although the relaxation of vascular smooth muscle by β-receptor agonists had previously been thought to occur via activation of adenylylcyclase and production of cAMP (13, 16), there have now been recent reports to suggest that NO may also have an important function in promoting vascular smooth muscle relaxation induced by β-agonists (10–12, 21, 23, 26). For example, Rubanyi and Vanhoutte (24) reported that removal of the endothelium in canine coronary arteries attenuated isoproterenol-induced vascular smooth muscle relaxation. Other recent studies have shown that β-receptor-mediated vascular smooth muscle relaxation may involve release of endogenous NO from the endothelium in isolated vascular preparations (11, 12, 21, 26) and that NOS is induced by cAMP (15). In isolated vascular
ring preparations, isoproterenol has been shown to act through endothelial β-receptors to raise levels of cAMP, an effect that is attenuated by inhibition of NOS (11, 12). These investigations on the role of the endothelium in β-agonist-mediated vascular smooth muscle relaxation have been conducted in isolated vascular or cultured cell preparations (11, 12, 21, 26) or by injection in a regional vascular bed (10, 23, 26). In contrast, we investigated the effects of inhaled isoetharine HCl on bronchial arterial blood flow in the intact animal and found a rapid increase in Qbr. Furthermore, inhibition of NOS with intravenous administration of L-NAME not only decreased Qbr by ~80% but also resulted in further attenuation of isoetharine HCl-induced increases in Qbr (Fig. 3). These findings suggest that isoetharine HCl stimulates release of endogenous NO in the bronchial circulation, thus confirming the findings obtained in prior in vitro vascular preparation and cell culture studies (11, 12, 21, 26). The responses in Qbr after rechallenge with isoetharine HCl were comparable when isoetharine was given either 20 or 80 min after intravenous L-NAME, indicating that the effect of NOS blockade was of long duration. This is in accordance with findings by White et al. (28), who, in a preliminary study, showed that intravenous infusion of L-NAME in the dog produced an immediate fall in bronchial vascular conductance by ~50%, an effect that was sustained for 3 h.

To determine whether the inhibitory effect of L-NAME on isoetharine-induced bronchial vascular dilation depends on the increased baseline vascular tone, we compared the effect of L-NAME with that of phenylephrine, a potent α1-receptor agonist, which results in vasoconstriction without inhibition of NOS. We found that the vasodilatory response to isoetharine was not affected by concurrent treatment with phenylephrine despite a vascular tone comparable to that obtained with L-NAME. These results indicate that the effect of NOS blockade on isoetharine-induced bronchial arterial vasodilation appears to be independent of the baseline vascular tone. We observed that L-NAME, when administered via the intravenous route, caused a rapid increase in BVR, whereas the effect of L-NAME given by inhalation was rather modest and delayed in onset. There may be several explanations for this difference. First, the dose of nebulized L-NAME was one-third of that given intravenously, and it is possible that delivery of the nebulized agent to the airways was not complete. Furthermore, it is expected that L-NAME administered intravenously would have rapid contact with the vascular endothelium and would thus have a rapid effect on inhibition of NOS. In contrast, L-NAME given by inhalation may require additional time to penetrate the bronchial mucosa to ultimately reach the submucosal plexus. It is also possible that L-NAME, given by inhalation does not have a rapid effect on Qbr, compared with other inhaled agents such as isoetharine HCl or other β-agonists, because of its molecular structure and other chemical properties. However, although the effect of inhaled L-NAME on Qbr was of a smaller magnitude, the vasodilatory effects of inhaled isoetharine with subsequent challenge were nonetheless attenuated.

In this model, Qbr decreased with NOS inhibition because bronchovascular resistance increased and not because of alterations in the upstream or downstream pressures, since there were no appreciable changes from baseline values in Psa or Ppa after a period of stabilization. However, it is interesting to note that, immediately after intravenous infusion of L-NAME, Qbr first rose acutely before its subsequent precipitous drop (Fig. 4A). This rise in Qbr was associated with the transient drop in Psa, and it was only at ~4 min after completion of the infusion that Qbr decreased and Psa increased (Fig. 4B). Although we do not have a conclusive explanation for this phenomenon of transient increase in Qbr associated with a decrease in Psa after L-NAME, a few speculations can be made. It is conceivable that L-NAME caused a rapid release of NO from the endothelium and resulted in transient, generalized vasodilation before NO was blocked. Although it was previously thought that NO-related factors were only released after their formation (14), recent studies have now provided evidence for the presence of preformed stores of NO-containing factors in the endothelium (8, 9). Thus, in view of the rapidity and transient nature of the resulting vasodilation after intravenous L-NAME, it can be speculated that this agent by itself or in conjunction with β-agonists may have an initial effect on preformed stores of NO-containing factors. In this study, the vasodilatory effect on the bronchial vasculature was transient, since NO is rapidly inactivated by hemoglobin (20), and the profound vasoconstriction resulting from NOS inhibition then became evident.

In summary, we show that administration of isoetharine HCl, a β-adrenoceptor agonist, by inhalation in doses used clinically resulted in marked vasodilation in the bronchial circulation of the intact sheep. This increase in Qbr occurred in a dose-dependent manner and was attenuated by inhibition of NOS. These data also suggest that, in the bronchial vasculature, β-agonist-induced relaxation of vascular smooth muscle is partially mediated via synthesis of endogenous NO. It thus appears that both cAMP and cGMP pathways mediate β-agonist-induced bronchial vascular relaxation.

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