Effect of aerosolized acetylcholine on bronchial blood flow

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Charan, Nirmal B., Paula Carvalho, Shane R. Johnson, William H. Thompson, and S. Lakshminarayan. Effect of aerosolized acetylcholine on bronchial blood flow. J. Appl. Physiol. 85(2):432 –436, 1998.—We studied the effects of aerosolized acetylcholine on bronchial blood flow in six anesthetized sheep. Intravenous infusion of acetylcholine, at a dose of 2 µg/kg, increased bronchial blood flow from 45 ± 15 (SE) to 74 ± 30 ml/min, and vascular conductance increased by 76 ± 22%. In contrast, aerosolized acetylcholine at doses of 2 and 20 µg/kg decreased bronchial vascular conductance by ~10%. At an aerosolized dose of 200 µg/kg, the bronchial vascular conductance increased by ~15%, and there was no further increase in conductance when the aerosolized dose was increased to 2,000 µg/kg. Pretreatment of animals with a nitric oxide synthase inhibitor, Nω-nitro-L-arginine methyl ester hydrochloride, partially blocked the vasodilatory effects of intravenous acetylcholine and completely blocked the vasodilatory effects of high-dose aerosolized acetylcholine. These data suggest that aerosolized acetylcholine does not readily penetrate the vascular wall of bronchial circulatory system and, therefore, has minimal vasodilatory effects on the bronchial vasculature.

bronchial circulation; airway circulation; nitric oxide

ACETYLCHOLINE IS A POTENT VASODILATOR when infused into the systemic circulation. However, Furchgott and Zawadzki (7) found that, in the absence of endothelium, acetylcholine is a vasoconstrictor. This finding eventually led to the discovery that acetylcholine-induced vasodilation is mediated through synthesis of nitric oxide by the endothelial cells. Similar to other vascular beds, it has also been well established that infusion of acetylcholine into the bronchial artery results in pronounced dilation in the bronchial vascular bed (11, 12).

Compared with most other vascular beds, bronchial circulation is somewhat unique because the vasoactive agents can be administered into the vascular bed either by intravascular infusion, whereby they first come in contact with the endothelium, or by aerosol whereby these agents first come in contact with the vascular adventitia and then penetrate the vascular wall before reaching the endothelium (1). Recently, there has been some interest in studying whether acetylcholine, when delivered to the bronchial circulation by inhalation, has similar effects compared with when it is given via the intra-arterial route. Recently, it was found that comparable doses of acetylcholine caused bronchial arterial vasodilation only with intravenous administration, whereas aerosolized acetylcholine did not cause vasodilation (12). This is in contrast to some other preliminary studies in which Lakshminarayan et al. (9) as well as Parsons et al. (10) found that aerosolized methacholine (also a cholinergic agonist) resulted in significant increases in bronchial blood flow. Furthermore, in a preliminary study, it has been found that aerosolized methacholine decreased bronchial blood flow by ~14% (8). The reasons for this discrepancy are not known, although one possible explanation could be that the effect of these agents on bronchial circulation is dose dependent. Therefore, we hypothesized that aerosol administration of acetylcholine, in relatively large doses, may result in penetration of some of the drug through the vascular wall into the intravascular compartment, causing vasodilation through endothelium-dependent mechanisms. On the other hand, smaller doses may have a direct effect only on the vascular smooth muscle, resulting in vasoconstriction. Thus in this project we studied the dose-dependent effects of aerosolized acetylcholine on bronchial blood flow.

METHODS

Surgical preparation. Six adult sheep of mixed breed were fasted for 24 h and then sedated with xylazine (0.25 mg/kg) ~30 min before surgery. After induction of anesthesia with intravenous injection of 5–10 ml of 5% pentobarbital sodium, sheep were intubated and connected to an anesthesia machine (Ohmeda Anesthesia System Excel 210, Madison, WI). Anesthesia was maintained with 1–2.5% halothane. A gastric tube was passed through the esophagus into the stomach to continuously drain the rumen. The animals were placed in a right lateral decubitus position, and a left thoracotomy was performed through the fifth intercostal space. The main pulmonary artery was dissected, and, depending on the size of the pulmonary artery, a 16- or 24-mm ultrasonic flow probe (Transonic Systems, Ithaca, NY) was placed around the pulmonary artery to monitor cardiac output. The bronchial artery was dissected, and a 2-mm ultrasonic flow probe was placed around the common bronchial branch of the bronchoscopic artery to continuously measure the bronchial blood flow. Although these flow probes tended to fit snugly around the pulmonary and the bronchial arteries, if a satisfactory flow signal was not obtained from the flow probes, Lectron II Conductivity Gel (Pharmaceutical Innovations, Newark, N J) was used as an acoustical couplant to improve the flow signals. Both flow probes were then connected to a dual-channel blood flowmeter (T201, Transonic Systems) for
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simultaneous recording of cardiac output and bronchial blood flow. Proper placement of the bronchial arterial flow probe was confirmed by administering 100 parts/million of nitric oxide by inhalation for 3 min and documenting the flow probe returned to baseline after discontinuation of nitric oxide, a technique that has been previously described (2). After this, the left lung was reexpanded and the chest was closed. A catheter was placed through the left jugular vein for measurement of pulmonary arterial pressure. The height of the left atrium was used as a zero reference for all hemodynamic measurements. All hemodynamic parameters, including signals from both flow probes, were continuously recorded on a multichannel recorder (model 2107-8890-00, Gould, Cleveland, OH).

The sheep were ventilated with a tidal volume of 10 ml/kg, and the respiratory rate was kept between 12 and 16 breaths/min to maintain an arterial Pco2 of ~40 Torr. The animals were also given supplemental oxygen to keep the arterial Po2 around 100 Torr. After initial ventilator adjustments to achieve the above blood gases, intermittent blood-gas analyses (at least once every hour) were performed to ensure that satisfactory blood gases were maintained throughout the experiment.

Calculation of bronchial vascular conductance. We first calculated bronchovascular resistance (BVR) as described previously (5). Briefly, we used the following equation:

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BVR = \frac{\text{mean systemic arterial pressure} - \text{mean pulmonary arterial pressure}}{\text{bronchial blood flow}}
\]

Bronchial vascular conductance was calculated by using the inverse of BVR. These calculations were made for each data point, and then we calculated percent change in bronchial vascular conductance by using pretreatment values (time 0) as control for each experimental condition.

Protocol. Through a Tee adaptor, a small-volume nebulizer (Saltor Laboratories, Arvin, CA) was connected between the endotracheal tube and the ventilator tube for nebulization of acetylcholine. The dose of acetylcholine bromide (Sigma Chemical, St. Louis, MO) was progressively increased in a stepwise fashion from 2 to 20 to 200 to 2,000 µg/kg. Each dose of acetylcholine was diluted in 5 ml of saline. With an oxygen tubing, the nebulizer was connected to an oxygen cylinder, and the solution was nebulized for 8 min at an oxygen flow rate of 8 l/min to nebulize the acetylcholine solution as much as possible. However, even with this 8-min nebulization period, some solution was always left behind in the nebulizer. Therefore, to estimate the dose of acetylcholine that was actually nebulized, the nebulizer was weighed before and after nebulization of acetylcholine solution. The effect of acetylcholine on bronchial blood flow was measured for 10 min after completion of each dose. At least 30 min after completion of the above protocol, a bolus injection of acetylcholine (2 µg/kg in 10 ml of saline) was given into a peripheral vein, and the effect of the drug on the bronchial blood flow was monitored for 5 min.

Because aerosolized acetylcholine could be rapidly inactivated by cholinesterase present in the airways, in three sheep we tested the effects of acetylcholine and edrophonium chloride, an anticholinesterase agent, administered together by aerosolization. For this segment of the study, we first administered edrophonium chloride (10 mg in 2.5 ml saline) by aerosolization for 3 min and studied its effects on bronchial vascular conductance. This was followed by administration of acetylcholine (20 µg/kg) and edrophonium chloride (10 mg) mixed together in 2.5 ml of saline, and the solution was aerosolized for 3 min.

To study whether inhibition of nitric oxide synthesis has any effect on vascular responses induced by aerosolized acetylcholine, in four animals a nitric oxide synthase inhibitor, N-nitro-l-arginine methyl ester hydrochloride (l-NNAME; Sigma Chemical), was given intravenously over a 1-min period, at a dose of 30 mg/kg diluted in 20 ml saline. After physiological parameters had stabilized (~20 min), we again infused 2 µg/kg acetylcholine intravenously and monitored physiological parameters. This was followed by a single dose of 2,000 µg/kg administered by aerosolization as described above.

Statistics. A one-way analysis of variance for repeated measures was used to compare changes in physiological parameters, and Dunnett’s test was utilized to compare baseline values (time 0) with the effect of the drug for each dose. A P value of <0.05 was regarded as significant. The data are presented as means ± SE.

RESULTS

All sheep were adult and nonpregnant females with a mean weight of 77.67 ± 3.2 kg. The control bronchial blood flow was 47 ± 13 ml/min, and there was a wide variation in bronchial blood flow among sheep. In one sheep, the baseline bronchial blood flow was as high as 100 ml/min, which resulted in wide SEs. Only ~25% of the total dose of acetylcholine in the nebulizer was actually nebulized (Table 1). Thus, at a dose of 2,000 µg/kg, we were able to deliver only 579 ± 170 µg/kg of acetylcholine. However, the exact amount of dose deposited on the airway mucosa could not be determined in this study.

As shown in Fig. 1, inhalation of acetylcholine (2 and 20 µg/kg) resulted in a ~10% decrease in bronchial vascular conductance (Fig. 1). With higher nebulized doses of acetylcholine, the bronchial vascular conductance tended to increase, and, at 200 µg/kg, the bronchial vascular conductance increased by ~15%. There was no further increase in bronchial vascular conductance when 2,000 µg/kg of acetylcholine were given by inhalation. However, it should be noted that the control bronchial blood flow, before administration of acetylcholine, was 47 ± 11 ml/min, and, after the last dose of 2,000 µg/kg, it was 61 ± 18 ml/min, representing a 30% increase in flow. This is in contrast to intravenous acetylcholine, which resulted in a transient fall in blood pressure associated with a rapid increase in bronchial blood flow in all animals, and, at 5 min after the infusion, bronchial vascular conductance was still 76% higher than the control value (P < 0.05). However, the magnitude of response to acetylcholine among sheep.
was variable, with some sheep showing marked increases in bronchial blood flow while others showed only modest increases.

Aerosolization of edrophonium chloride resulted in a decrease in bronchial vascular conductance by 20 ± 2%. When the mixture of edrophonium chloride and acetylcholine was administered together, the bronchial vascular conductance again decreased by 19 ± 2%. There were no significant changes in systemic arterial pressure and cardiac output.

With intravenous L-NAME, even though systemic arterial pressure increased, bronchial blood flow decreased to 27 ± 8 ml/min. With repeat dose of intravenous acetylcholine after L-NAME, there was only a 22% increase in bronchial vascular conductance. In contrast, after L-NAME, repeat administration of aerosolized acetylcholine (2,000 µg/kg) resulted in a 10% decrease in bronchial vascular conductance.

**DISCUSSION**

This study demonstrates that, compared with intravenous infusion, aerosolized acetylcholine does not cause a similar degree of vasodilation in the bronchial circulation, even when relatively high doses of acetylcholine are used. These findings are in agreement with those of Scuri et al. (12), who also measured bronchial blood flow in sheep by using color-coded microspheres. With this technique they were able to measure the blood flow going to airway mucosa as well as that part of the flow that supplies the bronchial wall. With use of this preparation, they found that, when 20 mg/ml of

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**Table 1. Effects of aerosolized and intravenous infusion of acetylcholine on bronchial blood flow**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Time, min</th>
<th>Dose</th>
<th>Q˙br, ml/min</th>
<th>CO, l/min</th>
<th>Psa, mmHg</th>
<th>Ppa, mmHg</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACh (2 µg/kg)</td>
<td>0</td>
<td>0.52 ± 0.80</td>
<td>47 ± 11</td>
<td>4.36 ± 0.33</td>
<td>88 ± 3</td>
<td>9 ± 1</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>µg/kg</td>
<td>46 ± 12</td>
<td>4.21 ± 0.32</td>
<td>88 ± 4</td>
<td>9 ± 1</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>µg/kg</td>
<td>45 ± 14</td>
<td>4.23 ± 0.40</td>
<td>90 ± 3</td>
<td>9 ± 1</td>
</tr>
<tr>
<td>ACh (20 µg/kg)</td>
<td>0</td>
<td>4.84 ± 1.37</td>
<td>46 ± 12</td>
<td>4.25 ± 0.32</td>
<td>88 ± 4</td>
<td>9 ± 1</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>µg/kg</td>
<td>43 ± 11</td>
<td>4.13 ± 0.32</td>
<td>87 ± 5</td>
<td>12 ± 1</td>
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<tr>
<td></td>
<td>10</td>
<td>µg/kg</td>
<td>42 ± 14</td>
<td>4.18 ± 0.39</td>
<td>89 ± 4</td>
<td>12 ± 1</td>
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<td>ACh (200 µg/kg)</td>
<td>0</td>
<td>46.2 ± 7.19</td>
<td>46 ± 11</td>
<td>4.14 ± 0.30</td>
<td>85 ± 5</td>
<td>12 ± 1</td>
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<tr>
<td></td>
<td>5</td>
<td>µg/kg</td>
<td>49 ± 14</td>
<td>4.16 ± 0.35</td>
<td>85 ± 5</td>
<td>12 ± 1</td>
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<tr>
<td></td>
<td>10</td>
<td>µg/kg</td>
<td>54 ± 19</td>
<td>4.26 ± 0.43</td>
<td>91 ± 3</td>
<td>12 ± 1</td>
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<tr>
<td>ACh (2,000 µg/kg)</td>
<td>0</td>
<td>579 ± 170</td>
<td>57 ± 18</td>
<td>4.23 ± 0.41</td>
<td>83 ± 5</td>
<td>12 ± 1</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>µg/kg</td>
<td>60 ± 16</td>
<td>4.37 ± 0.29</td>
<td>86 ± 6</td>
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<tr>
<td></td>
<td>10</td>
<td>µg/kg</td>
<td>61 ± 18</td>
<td>4.39 ± 0.37</td>
<td>87 ± 4</td>
<td>12 ± 1</td>
</tr>
<tr>
<td>ACh (2 µg/kg iv)</td>
<td>0</td>
<td>2 µg/kg</td>
<td>45 ± 15</td>
<td>4.46 ± 0.40</td>
<td>85 ± 5</td>
<td>8 ± 1</td>
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<tr>
<td></td>
<td>5</td>
<td>74 ± 30*</td>
<td>4.19 ± 0.36</td>
<td>80 ± 5</td>
<td>8 ± 1</td>
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<tr>
<td>L-NAME (30 mg/kg iv)</td>
<td>0</td>
<td>30 mg/kg</td>
<td>65 ± 32</td>
<td>4.21 ± 0.36</td>
<td>82 ± 6</td>
<td>8 ± 1</td>
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<tr>
<td></td>
<td>10</td>
<td>27 ± 9*</td>
<td>3.38 ± 0.42</td>
<td>121 ± 14</td>
<td>11 ± 1</td>
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<tr>
<td>ACh (2 µg/kg iv)</td>
<td>0</td>
<td>25 µg/kg</td>
<td>27 ± 8</td>
<td>3.27 ± 0.45</td>
<td>101 ± 14</td>
<td>10 ± 1</td>
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<td></td>
<td>5</td>
<td>27 ± 8</td>
<td>3.28 ± 0.48</td>
<td>95 ± 5</td>
<td>10 ± 2</td>
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<tr>
<td>ACh (2,000 µg/kg)</td>
<td>0</td>
<td>394 ± 130</td>
<td>27 ± 9</td>
<td>3.45 ± 0.31</td>
<td>91 ± 5</td>
<td>13 ± 1</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>24 ± 8</td>
<td>3.30 ± 0.38</td>
<td>90 ± 7</td>
<td>10 ± 1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>24 ± 8</td>
<td>3.62 ± 0.65</td>
<td>90 ± 5</td>
<td>10 ± 1</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE. Dose, actual dose that was nebulized; Q˙br, bronchial blood flow; CO, cardiac output; Psa, systemic arterial pressure; Ppa, pulmonary arterial pressure; L-NAME, N\textsubscript{\textit{\textn}}-nitro-L-arginine methyl ester. * P < 0.05 compared with time 0 value.

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**Fig. 1.** Effect of varying doses of aerosolized (solid bars) and a single dose of intravenous (hatched bars) acetylcholine on bronchial vascular conductance. Bronchial vascular conductance is shown as percent change from pretreatment value (time 0) for each dose.
Acetylcholine were aerosolized for 1 min, there was a 9% decrease in bronchial blood flow. On the other hand, an intravenous bolus of 2 μg/kg resulted in a 290% increase in bronchial blood flow. They concluded that intravenous but not aerosolized acetylcholine increases bronchial blood flow in the bronchial mucosa as well as in the bronchial wall. In contrast, we used a flow probe on the common bronchial branch of the bronchoesophageal trunk that supplies the entire bronchial tree beyond the tracheal bifurcation. Thus, with this technique, we were able to measure bronchial blood flow beyond the tracheal bifurcation only and not in the trachea. Because these animals were intubated, aerosolized acetylcholine must have been deposited on the distal trachea and the bronchi. However it is possible that, at higher dosages, some of the drug did get deposited on the alveolar epithelium and got absorbed into the systemic circulation. In this study we did not measure the aerosol mass, and therefore, we are unable to estimate the amount of drug that could have reached the alveolar space.

In the airways, the bronchial circulation forms a plexus in the peribronchial connective tissue and another in the bronchial submucosa (3). We used a flow probe that measures the bronchial blood flow in both of these plexuses. The aerosolized drugs are likely to penetrate only the submucosal plexus, whereas when the drugs are given by intravenous infusion they affect both the peribronchial as well as the submucosal plexuses. Therefore, if the proportion of blood flow supplying the submucosal plexus is relatively small, we would not expect to see much increase in total bronchial blood flow with aerosolized acetylcholine. This cannot be an explanation for our findings because it has been shown by using microsphere techniques that over 70% of the total bronchial blood flow goes to the submucosal plexus (12).

We had reasoned that, if higher doses of aerosolized acetylcholine were used, we might be able to produce an effect on bronchial vasculature that is comparable to intravenous infusion. However, we found that even substantially higher doses of acetylcholine produced only a minimal increase in bronchial vascular conductance. Although it is not apparent from this study why acetylcholine does not readily penetrate the vessel wall to reach the vascular endothelium and cause an increase in bronchial blood flow by stimulating nitric oxide synthesis, a few potential explanations can be speculated. It is possible that acetylcholine deposited in the airways is rapidly hydrolyzed by cholinesterase present in the airways, and, therefore, only a small amount of active drug is available to act on the endothelium. However, if this were the case, we should have seen increasingly pronounced vasodilator effects as the dose of acetylcholine was increased 1,000-fold. Furthermore, use of edrophonium chloride (an anticholinesterase drug) did not enhance the vasodilatory response of aerosolized acetylcholine, which also suggests that it is unlikely that acetylcholine was rapidly inactivated by anticholinesterase. The other possibility is that the bronchial mucosa and the wall of bronchial vasculature provide a barrier to the acetylcholine molecule, and, therefore, it is unable to enter the blood vessel. Indeed, there is some evidence that the adventitia of the pulmonary artery can be a barrier to certain molecules such as nitric oxide (13). On the other hand, Scuri et al. (12) found that inhaled acetylcholine does increase the lung resistance, providing the evidence that this drug is capable of penetrating the bronchial mucosa. It is interesting that, at a dose that is comparable to the one aerosolized by Scuri et al., we actually observed a decrease in bronchial vascular conductance. This suggests that, even at comparatively smaller doses, acetylcholine is able to penetrate the bronchial mucosa and directly act on the vascular smooth muscle causing vasoconstriction. The decrease in bronchial conductance seems to indicate that acetylcholine did indeed reach the bronchial vascular smooth muscle. This finding also seems to indicate that it must be the muscular layer of the bronchial vasculature that acts as a barrier and prevents acetylcholine to penetrate the vessel wall.

Intravenous injection of acetylcholine not only caused a decrease in systemic arterial pressure but also resulted in marked increase in bronchial vascular conductance. On the other hand, treatment with l-NAME caused an increase in systemic arterial pressure associated with a decrease in bronchial vascular conductance. These data suggest that nitric oxide synthesis plays an important role in regulation of bronchial vascular tone. As expected, l-NAME also partially blocked the increases in bronchial vascular conductance caused by intravenous infusion of acetylcholine. However, it is also interesting that, after l-NAME, aerosolization of acetylcholine in high doses, instead of increasing bronchial blood flow, resulted in a decrease in bronchial blood flow, suggesting that the vasodilatory effects of aerosolized acetylcholine were indeed mediated through the synthesis of nitric oxide in the vascular endothelium.

In summary, this study demonstrates that, compared with intravenous route, aerosolized acetylcholine in smaller doses constricts the bronchial vasculature, and, when given in very high doses, it has only a modest vasodilatory effect that is mediated through the synthesis of nitric oxide.

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